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A NEW ULTRAVIOLET SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF D-TUBOCURARINE CHLORIDE IN PLASMA*

MRS. BERNICE THEISSEN ELERT, B.S., M.T. (ASCP)
Charles T. Miller Hospital, St. Paul, Minn.†

The term curare is an Indian word meaning "poison" and has been applied to the large variety of arrow poisons which were formerly used by primitive Indians in South America. Tubocurarine is the principal alkaloid of *Chondodendron tomentosum* and those related species, the stem bark of which yields extracts commonly referred to as tube curare. In 1898, Boehm isolated from this tube curare, the highly active quaternary ammonium compound, tubocurarine.

The characteristic action of curare of the tubocurarine type is muscular relaxation induced by the blocking of nerve impulses between the nerve fiber and the muscle fiber supplied by it. The mechanism of action apparently involves prevention of the acceptance by the muscle of the mediating substance, acetylcholine. It is used in surgical procedures as a supplement to anesthetic agents in order to provide increased muscular relaxation with smaller doses of anesthetic agents, to soften convulsions in electroshock therapy, to reduce muscle spasm in fractures, and in the diagnosis of myasthenia gravis. When the drug is injected intravenously in aqueous solution, the physiologic effects appear in 2-3 minutes.

In recent years, tubocurarine chloride has found wide spread use as an adjunct to anesthesia, but there is still much work to be done to solve the problems of the duration of action, rate of destruction or rate of disappearance, and correlation of blood level with paralysis. The information provided in the literature by the present available methods for analysis is incomplete, and the problem has been complicated by the extremely low concentration of drug attained in the plasma. Since d-tubocurarine is a

* Second ASMT award. Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.
† Division of Biochemistry, Department of Pathology. Funds for this study were supplied by a generous contribution from the Lewis and Annie F. Paper Foundation Fund.

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In recent years, tubocurarine chloride has found wide spread use as an adjunct to anesthesia, but there is still much work to be done to solve the problems of the duration of action, rate of destruction or rate of disappearance, and correlation of blood level with paralysis. The information provided in the literature by the present available methods for analysis is incomplete, and the problem has been complicated by the extremely low concentration of drug attained in the plasma. Since d-tubocurarine is a

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very potent drug and one which possesses great potentiality for harm if improperly used or dispensed, the advantages of simple and readily performed assay procedures for analytical control purposes are apparent.

In 1949 Klein and Gordon,[5] studying solutions in bulk and in sterile containers, presented three methods of identification of d-tubocurarine chloride in aqueous solution according to properties of the drug. The preferred method was based on the drug's absorption of light in the ultraviolet region, which could be measured spectrophotometrically. These authors presented a study of the ultraviolet absorption curve of the salt in aqueous solution and demonstrated an absorption maximum at 280-281 mu. This method forms the basis of measuring the drug in plasma, after appropriate extraction.

In 1950[6] the first chemical method of plasma determination of d-tubocurarine chloride was reported. The principle was originally employed by Brodie and Udenfriend[7] for organic bases, that is, extraction of the alkaloid into an organic solvent and subsequent complex formation with methyl orange. D-tubocurarine can only be extracted from a potassium iodide solution at pH 10 into organic solvents. Quinn and Woislawski[6] demonstrated that other salts were unsatisfactory because they caused the formation of emulsions between the plasma and solvent, gave high blanks, or failed to result in complete recovery. The methyl orange complex formation was read in a photoelectric colorimeter at 540 mu. using the organic (ethylene dichloride) as a blank. However, in the plasma there are ethylene dichloride soluble substances which react with methyl orange. These workers found the blank obtained with 1 ml. of rabbit's plasma was equivalent to .06 microgram of d-tubocurarine. This work was conducted to correlate plasma levels of d-tubocurarine chloride with the dose required to produce "head drop" in rabbits.

Porter and Silber[3] working with dog plasma also presented a modification of the original Brodie and Udenfriend[7] dye method. They were able to realize increased sensitivity by the continuous transference of methyl orange from a buffer to dilute alkali by means of a solution of the drug in an organic solvent. This method was time consuming and at one point required shaking of the two phases for twenty-four hours. This dye method for determination of basic drugs lacks specificity but has attained wide popularity because of its simplicity and general applicability.

Mitchell and Clark[8] in a general method for the determination of quaternary ammonium compounds in which at least one chain is 4 carbons or longer, applied brom phenol blue for the dye complex in the estimation of acetylcholine, hexamethonium,

tetraethylammonium, neostigmine, and d-tubocurarine in certain media. This paper brought to light the realization that other normally present quaternary ammonium compounds in blood may interfere with the chemical assay of d-tubocurarine chloride.

Marsh[4] in his work used labeled C_{14} d-tubocurarine on rats, guinea pigs, rabbits, and dogs. He traced renal excretion, liver and tissue uptake, and observed that the drug was held in extravascular compartments and could be mobilized back in dehydration and electrolyte or fluid shift.

Pittinger, Morris, and Cullen[10] adopted the chemical method of Quinn and Woislawski[6] in their studies of d-tubocurarine chloride levels in human plasma after intravenous injection during anesthesia. A number of experimental difficulties were encountered with this method. Lack of specificity of the procedure and the minute quantities of drug present in the plasma made the analytical technique very difficult. It was necessary that all laboratory glassware be washed with detergent and water, rinsed, washed with sodium dichromate sulfuric acid cleaning solution and thoroughly rinsed with tap, distilled and pyrogen-free water. All syringes and needles were likewise treated, but were then additionally rinsed with isopropyl alcohol and ether separately and allowed to dry by evaporation before autoclaving.

The Quinn and Woislawski method[6] was also used by Cohen and workers[13] in their studies. The same washing procedure for laboratory glassware as above was utilized and in addition, all glassware was baked for 30 minutes at 250° F. as a means of further insuring clean glassware. In our work on placental transmission of tubocurarine in humans[13] many difficulties were encountered which made the methyl orange method unsuitable. The method was extremely time-consuming and fraught with many extrinsic and intrinsic errors. On the former, as noted by Mitchell and Clark, many basic compounds react with methyl orange. Hence the methyl orange method measures not only tubocurarine but other basic substances, normally present in blood or used in conjunction with tubocurarine. The long time required for each analysis precluded the study of many pressing problems with this important drug. Hence a more specific method was sought.

The purpose of this article is to present a rapid and specific method for extraction of d-tubocurarine chloride from human plasma and subsequent measurement by ultraviolet spectrophotometry.

Preparation of Reagents

The ethylene dichloride, purified, purchased from Eimer and Amend, was further purified by either redistilling from a water bath in all glass distillation apparatus and collecting the distillate at 83° C. or by successive washings with 0.01 N hydrochloric acid. Each successive acid extract was read in the Beck-

man DU at 280.5 mu, and the extraction continued until the Beckman readings were constant. If the EDC was cloudy, it was cleared with sodium sulfate.

The working potassium iodide-glycine buffer, pH 10, was prepared according to Quinn and Woislawski by mixing 6 ml. of 0.1 M glycine solution[1] with 4 ml. of 0.1 N sodium hydroxide. To 10 ml. of this, 12.8 grams of reagent grade potassium iodide were added and dissolved. The 0.1 M glycine solution is stable in the refrigerator but the working KI-buffer is not, and the mixture should be prepared fresh daily.

The standard d-tubocurarine chloride pentahydrate aqueous solution (1 ml. = 25.4 micrograms)[2] was made from the colorless crystalline powder as defined in the article by Klein and Gordon.[5] The standard is stable at refrigerator and room temperatures in a pyrex glass stoppered bottle.

Procedure

A series of standards, 1 to 6 ml. were pipetted into 100 ml. pyrex glass stoppered bottles. Following the addition of 1.0 ml. of KI buffer, pH 10, and 10 ml. of purified redistilled ethylene dichloride, the mixture was shaken mechanically for 5 minutes. The samples were then transferred into 15 ml. pyrex centrifuge tubes and the phases separated by rapid centrifugation for 2-3 minutes. The aqueous phase was then suctioned off and discarded, and 7 ml. of the organic phase were immediately pipetted into 100 ml. pyrex glass stoppered bottles containing 5 ml. of 0.01 N HCl. These bottles were shaken mechanically for five minutes. At the end of this time, the samples were poured into clean 15 ml. pyrex centrifuge tubes and centrifuged for five minutes. The upper aqueous layer was then carefully aspirated off into a clean test tube, and a slow current of air was directed into it for three to five minutes to rid the 0.01 N HCl containing the salt of any residual ethylene dichloride. The samples were then ready for measurement. A method blank was run to cancel out any interference from reagents. This blank served as a reference solution and was used to set the instrument at 100% transmission before reading the standard samples.

All plasma samples were from postoperative hospital patients to whom tubocurarine had been administered. Seven ml. of oxalated plasma were pipetted into the 100 ml. pyrex glass stoppered bottles plus 1.0 ml. KI buffer and 10 ml. ethylene dichloride and carried through the procedure exactly the same as the standards. The plasma blank consisted of 7 ml. of plasma drawn before the d-tubocurarine chloride was given, and then the sub-

[1] 0.1 M glycine was prepared by weighing out 7.505 grams of glycine and 5.85 grams of NaCl into a 1000 ml. volumetric flask and diluting to volume with distilled water.

[2] The standard d-tubocurarine chloride pentahydrate aqueous solution was provided by Endo Laboratories, Inc., Richmond Hill, New York.

sequent samples were drawn at specific times after administration of the drug. This plasma blank was used to set the instrument at 100% transmission when reading the optical density of the plasma levels.

For spectrophotometric absorption in the ultraviolet region, the Beckman DU Spectrophotometer with photomultiplier attachment and ultraviolet light source was used. The operation of the instrument has been described in detail (15, 16). The settings optimal for tubocurarine chloride determination in this laboratory were:

Wave Length .....280.5 mu.
Slit Width ..... .08 mm.
Selector Switch ..... Check for Blanks, 1.0 for samples
Resistor ..... 22 megohms

The photomultiplier tube was positioned, and the photomultiplier switches were set at sensitivity 4 and zero suppression 0. When the photomultiplier tube is employed, the sensitivity may be regulated, not only with the sensitivity knob on the monochromator, but also by changing the power passing into the photomultiplier tube. The slit width was selected in accordance with the best resolving power. In general, the narrowest slit giving adequate readings should be used. These settings, however, are arbitrary and must be determined for each instrument. For all analyses a matched pair of quartz rectangular cells with a 1 cm. absorption path were used. During the initial work on this technique, the optical density (—log T) was determined from 240-300 mu. The absorption peak was obtained at 280.5 mu. as expected and all subsequent determinations were at 280.5 mu. The entire procedure took from 1-2 hours depending upon how many samples were run.

To determine applicability of the Beer-Lambert law, measure-

TABLE I
Relationship Between Concentration of d-Tubocurarine Chloride and Extinction

d-Tubocurarine Chloride Pentahydrate in 0.01 N HCl Micrograms	Optical Density	$K_{1\ cm.\ 280.5\ m\mu}$
12.7	.013	1.02
25.4	.025	.985
25.4	.025	.985
23.8	.023	.970
25.4	.025	.985
50.8	.0508	1.00
50.8	.0508	1.00
50.8	.051	1.00
50.8	.052	1.02
76.2	.077	1.01
76.2	.077	1.01
127.0	.128	1.01
127.0	.127	1.00
127.0	.129	1.02
152.4	.152	.998
152.4	.153	1.00
152.4	.153	1.00
	Average...	1.0008

ments were made of a series of standard solutions containing 25 to 150 micrograms of the pentahydrate. The data as tabulated in Table I was derived by application of $K = \frac{L}{C}$ where C is the concentration of the standard, L the optical density, and K the constant at 280.5 mu, 1 cm. Since C is in micrograms and not milligrams, the formula is $K = \frac{1000\,L}{C}$ e.g. from Table I.

$\frac{1000 \times 0.013}{12.7} = 1.02$, $\frac{1000 \times 0.025}{25.4} = .985$, etc.

The average value for E, 1%, $K_{1\text{ cm. }208.5\text{ mu,}}$ of 1.0008 (range .970—1.02) shows that the Beer-Lambert law holds for d-tubocurarine chloride at 280.5 mu. The per cent deviation from the average $K_{1\text{ cm. }208.5\text{ mu,}}$ was not unexpected since the method required several steps. On repeated optical density measurements of standards over the period of four days, there was no change in value. The d-tubocurarine chloride in 0.01 N HCl was apparently very stable.

From the average K value derived in Table I, the plasma concentrations were calculated as follows:

If 7 ml. of plasma were used:

$$\frac{\text{O.D. of plasma sample}}{K_{1\text{ cm. }280.5\text{ mu.}} \times 7} = \text{micrograms d-tubocurarine chloride per ml. of plasma}$$

Plasma samples stored in the refrigerator were also carried through the entire procedure on successive days and there was no change in optical density values.

When utilizing the 0.01 N HCl washed ethylene dichloride for analyses, the O.D. readings were somewhat higher on standards and plasma samples, but since the Beer-Lambert Law held, linearity was realized, and the error was cancelled out. This indicated that standards and blanks should be included in each assay.

Dutcher[14] in his isolation of tubocurarine chloride made a specific point of converting, as soon as possible, the free base to its salt. For this reason the time between the first step, addition of plasma and standards to KI buffer and ethylene dichloride, and the second shaking of 0.01 N HCl and ethylene dichloride must be very short. Only two samples were handled at a time to insure rapid manipulation. Slow manipulation gave results up to 30% lower than anticipated values.

The choice of solvents for ultraviolet is far more limited than for the visual range due to the fact that many organic solvents are more or less completely absorbent over the greater part of the ultraviolet range. The transparency of the 0.01 N HCl used as the final solvent for the drug was sufficient so that the machine sensitivity over the range used was great enough for

dependable and accurate results to be obtained. The 0.01 N HCl was kept in a pyrex container to prevent storage effects. All glassware was scrupulously clean since many factors which would not affect the curves of a visually absorbing solution will cause a good deal of interference and error in ultraviolet analyses. All glassware was washed with detergent and water, rinsed, washed in sodium dichromate sulfuric acid cleaning solution and thoroughly rinsed with tap and distilled water.

In order to exemplify reproducibility and recovery of d-tubocurarine chloride in the analyses of human plasma, each of six plasma samples was analyzed in duplicate with and without the addition of varying quantities of the drug as seen in Table II. The recoveries were excellent as shown by an average recovery of 100.3%.

TABLE II
Recovery of Varying Concentrations of d-Tubocurarine Chloride From Plasma

O.D. of Plasma Samples	O.D. of Plasma to Which Recovery Was Added	Difference	Mcg. of d-Tubo Cl		Recovery Percent
			Added	Recovered	
.030	.056	.026	25.4	26.0	102
.045	.070	.025	25.4	25.0	99
.003	.029	.026	25.4	26.0	102
.022	.072	.050	50.8	50.0	98
.019	.044	.025	25.4	25.0	99
.039	.065	.026	25.4	26.0	102

By means of the Beckman ratio-recording spectrophotometer,[3] it was possible to scan the ultraviolet, visible, and near-infrared from 200 to 3000 mu. in a thorough search of the entire spectrum to prove further the peak absorption of d-tubocurarine chloride. The reference solution used was 0.01 N HCl. The drug, in 0.01 N HCl, was found to have a noticeable absorption peak at 280-281 mu., no absorption in the visual, but a striking absorption peak in the near-infrared at 1525 mu. This latter observation will very likely lead to a more refined technique for analytical measurement. We intend to pursue this phase of study as soon as more adequate instruments are available. To prove the specificity of this new ultaviolet method for plasma extractions of d-tubocurarine chloride, possible interfering substances normally present in the plasma with similar chemical structure to the drug were also scanned in the ultraviolet region. These included histamine (no peak), creatine (no peak), acetyl choline (peaked at 238 mu.), sodium pentathol (peaked at 305 mu.), and adrenalin (peaked at 279-281 mu.). However, with concentrations of adrenalin present in the plasma (1:1,000,000,000), there was found to be no absorption peak perceptible. An attempt was made to quantitate results with this instrument using d-tubocurarine chloride, and a relative relationship was realized. The

[3] Courtesy of A. S. Aloe Company, Minneapolis, Minn.

instrument served to prove that measurement of d-tubocurarine chloride was being made at peak absorption and not on the side of the curve, and that substances normally occurring in plasma which can potentially form quaternary compounds were not interfering with quantitative results at 280.5 mu.

Summary

A rapid and simplified method for extraction of d-tubocurarine chloride from human plasma and measurement by ultraviolet spectrophotometry is presented. The ultraviolet measurement confirms the absorption peak of the drug at 280.5 mu.

The method was proven specific by use of the Beckman ratio-recording spectrophotometer. Recoveries of the drug from human plasma show reproducibility within 2%.

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REBOILING AND DILUTION AS FACTORS AFFECTING RESULTS IN THE FOLIN-WU DETERMINATION OF GLUCOSE*

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INTRODUCTION. The determination of the blood sugar level is probably done more often than any other routine biochemistry procedure; and of the numerous methods that have been developed, the Folin-Wu with its various modifications is still—apparently—the one preferred by most workers.

Since the first publication of this method by Folin and Wu[1], the original authors[2] and others have made continuous efforts to improve upon it; and today, with all its inherent limitations, its value as a rapid, dependable, diagnostic laboratory procedure cannot be overestimated.

In many procedures, the experienced worker learns to judge by the results of the daily runs over a period of time whether or not the method in use is giving misleading results; but in the case of blood sugar, the wide and rapid fluctuations in even normal individuals makes this type of control virtually impossible. Therefore, careful attention to all the variable features of the test becomes imperative.

This paper will cover two principle sources of error involved in this determination, with one other minor point mentioned briefly. It is likely that the first of these is the greater offender in causing deviations from the actual value†; but the other can cause sufficient error to make it worthy of correction.

It was recognized long ago that one of the chief drawbacks of the original method was the instability of the final color; and many of the modifications of the procedure were attempted in order to correct this defect.[3,4,5] Sunderman and Fuller[6] and Tonks[5] found boiling after the addition of the molybdic acid to be a satisfactory solution to the problem. (Sunderman and Fuller worked with the Benedict procedure, but the final colored compound is the same).

Before starting the work, a number of laboratory and instrument manuals and reference books were consulted; and, as shown by Table I, the essential steps were listed according to each source.

Of the thirteen sources listed, seven include color stabilization. Of the six that do not, four make no mention of the time factor in making the readings; the fifth states that they should be read "after 10 minutes," and the sixth "between 10 and 30 minutes."

In their selection of a filter or wavelength to be employed,

* Read before the First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June, 1956.

† "Actual value" refers, of course, to total reducing substances, which is what this test measures.

	Vol. filtrate (macro)	Vol. cu.sol.	boiling time	cooling time	vol.mo-lyb.sol	re-boil or no.	standing time	filter or WL
HAWK, OSER, SUMMERSON Practical Physiological Chem. 13 Ed.	1 ml.	2 ml.	8 min.	cool in running H_2O	2 ml.	no	not given	420
KOLMER, SPAULDING, ROBINSON Approved Lab. Technic 5 Ed.	1 or 2 ml.	2 ml.	8 min.	2-3 min in cold H_2O	2 ml.	no	after 10 min.	420
HILLER Practical Clinical Chemistry 1st printing	1 ml.	2 ml.	6 min.	3 min.	2 ml.	5 min.	within 30 min.	500
REINER Std. Methods of Clin. Chem. Vol. I	2 ml.	2 ml.	8 min.	Not given	2 ml.	2 min.	not given	420
VARLEY Practical Clin. Biochemistry 1st Ed.	1 ml.	2 ml.	8 min.	1-2 min in cold H_2O	2 ml.	2 min.	not given	680
METHODS FOR MED. LAB. TECHNICIANS. Dept. Army & Air Force	1 ml.	3 ml.of 2:3 dil	6 min.	3 min.H_2O at room temp	2 ml.	no	not given	520
FISTER Manual of Standardized Proc. for Spectrophotometric Chem.	2 ml.	1 or 2 ml.	8 min.	3 min.cold running H_2O	4 ml.	5 min.	after 2 min.	500
HELPER Manual of Clinical Laboratory Methods 4 Ed.	1 ml.	1 ml.	8 min.	3 min. H_2O at 10°C	2 ml.	5 min.	within 10 min.Keep cold until read	420
TODD SANFORD WELLS Clin.Diag. by Lab. Methods 12 Ed.	2 ml.	2 ml.	8 min.	cool in running H_2O	4 ml.	no	not given	not given
LEITZ HANDBOOK	2 ml.	2 ml.	8 min.	cool in running H_2O	2 ml.	5 min.	not given	520
KLETT-SUMMERSON CLINICAL MANUAL	2 ml.	2 ml.	6 min.	2-3 min in cold H_2O	2 ml.	no	between 10 & 30 min.	420
BAUSCH & LOMB MANUAL Methods & Calibrations	2 ml.	2 ml.	8 min.	2 min. in running H_2O	2 ml.	5 min.	not given	430
COLEMAN MANUAL OF CLINICAL METHODS	2 ml.	2 ml.	8 min.	in ice; time not given	2 ml.	no	not given	420

TABLE I

there is considerable disagreement. Generally, one of 420 mu seems to be the most popular, with most of the other preferences in the 500-520 range. This, of course, depends somewhat on the instrument and the size of the cuvette.

FORM OF PROCEDURE. A description of the actual procedures performed for these experiments will be given here in order to avoid having to describe them piecemeal later. One instrument was used throughout—a Coleman, Jr., spectrophotometer, with a cuvette of 19 x 150 mm. Two wavelengths were employed—420 and 515 mu. A stabilizing time of five minutes was used when this step was included. It was not possible from a standpoint of time to investigate other wavelengths, and the two minute boiling time specified by some authors. This latter point, has, however, been investigated by Tonks[5]*, who reports it to be as satisfactory as the five minute time.

Three standards, representing 100, 200, and 400 mg.% glucose, one non-fasting blood sample, and a synthetic control sample were used. The control consisted of some bovine albumin of the type used in Rh studies, diluted to normal protein level, to which glucose was added in a quantity of exactly 200 mg.%. Another non-fasting blood sample with added glucose was used for the experiment on dilution.

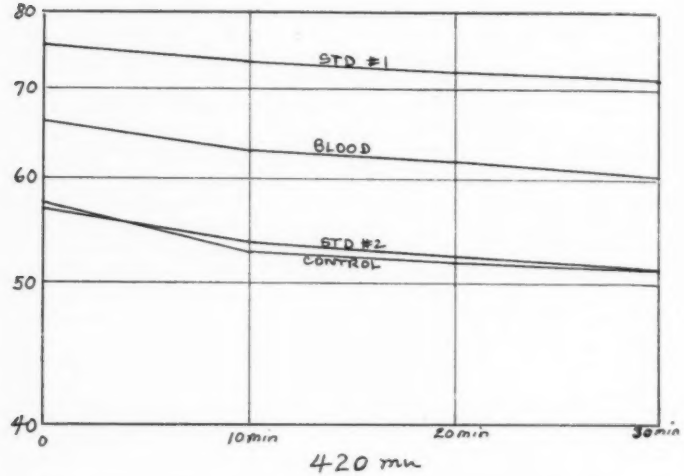
1. STABILITY OF COLOR. The first source of error to be considered was that of the color change that takes place on standing, if the color has not been stabilized by re-boiling.

Table II shows the color shift that takes place over a period of 30 minutes at the two wavelengths studied, when the color is not stabilized; and Table III shows the stabilizing effects of re-boiling. It will be noted that, at 420 mu, the shift is to the left in transmittance, or a continued increase in absorption of light. At this wavelength, the greatest change takes place within the first 10 minutes. At 515 mu, there is first a slight drop in transmittance within 10 minutes (or, as in the case of standard #1, no change at all) followed by a slight but continuous rise.‡ In one case, that of the control, the reading remains practically steady between 10 and 20 minutes, then begins its rise. At 420 mu, the average variation in transmittance over the 30 minute period amounts to 6 transmittance points. At 515, the greatest variation that could be found on one sample is 2½ transmittance points, which occur within the first 10 minutes.

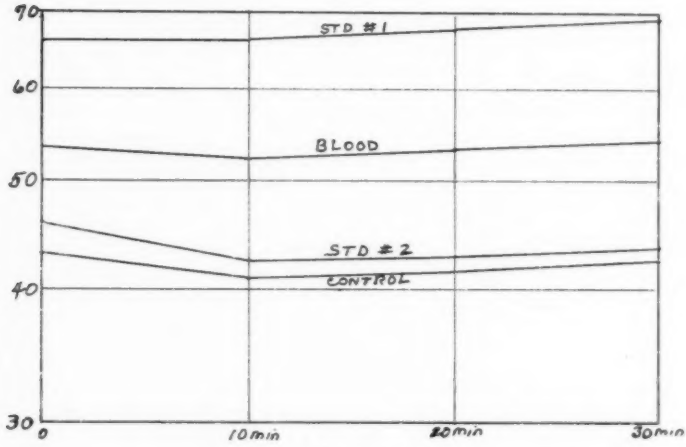
It is apparent that if color stabilization is not included, the preferred wavelength in this case and with this instrument would be closer to 515 than to 420 mu, as the time error is consider-

* Tonks also investigated a number of other modifications designed to stabilize the final color.

‡ Sunderman and Rasek[7] found the same effects, though with different wavelengths on a different instrument, in experimenting with color stabilization in the Benedict method; and also determined the wavelength at which the curves crossed—i.e., that at which no shift took place.



420 mμ



515 mμ

TABLE II

ably lessened. There is one other point to be noted here. If curves are prepared at the two wavelengths using both the non-stabilizing and the stabilizing techniques, it will be observed (Tables IV and V) that at 515 the curve after re-boiling is con-

siderably less steep than if not re-boiled. The reverse is true at 420 mu; re-boiling steepens the curve. In the first case, therefore, the readable color range is increased, and in the second it is decreased*, a factor which should be considered in the selection of a wavelength for use. This also is probably the place to mention the fact that obtaining an accurate curve without re-boiling is an extremely difficult procedure at best.

In order to give an idea of the possible error that could take place, the following calculations were performed: From the readings made in the preparation of Table II, the 30 minute reading (71) of standard #1 at 420 mu was accepted as the "standard" to be used for purposes of calculation. This reading was chosen because standards are usually read at the end of a run. The readings on the blood sample from this same series were as follows: Immediate reading, 66.; 10 minute reading, 62^2; 20 minute reading 61^1; and 30 minute reading, 60^1. Read against the standard, these readings would give the following results: Immediate, 121.5 mg.%; 10 minutes, 137 mg.%; 20 minutes, 143 mg.%; and 30 minutes, 147.5 mg.%.

2. DILUTION. The second source of error is that due to insufficient dilution of the sample or of the final color. If the color is too concentrated, it will be read on the extreme left end of the transmittance scale, where accuracy is considerably lessened. It is a commonly known fact that greatest accuracy is obtained by avoiding both extremes of the scale.[8,9,15] This is usually observed by the worker in the process of preparing his curves.

The problem of dilution has been discussed previously by Sister Mary Cyril[10] who found that the final color could be diluted before being read with results accurate within 3%. For this paper, a somewhat more detailed experiment was conducted.

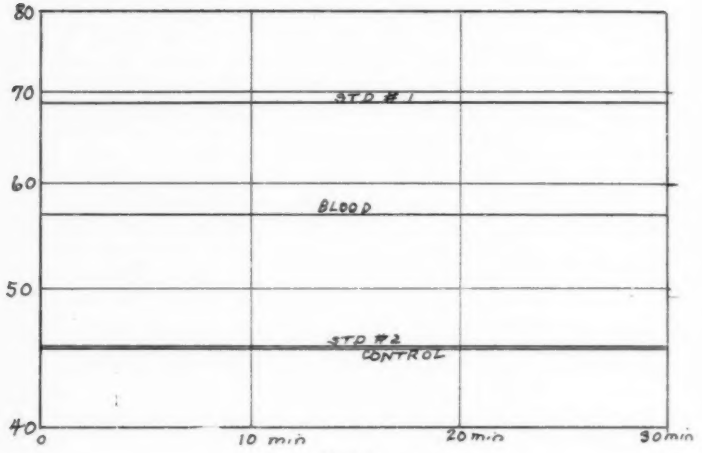
To determine when and where the dilution could take place, the following experiment was performed. A sample of blood was obtained from a normal donor (non-fasting, and collected about mid-morning) and to it was added approximately 300 mg.% glucose.

One portion of the blood was immediately diluted 1:2 with distilled water, and another portion 1:3. A third portion remained undiluted. Filtrates were prepared from each of these (diluted and undiluted) samples.

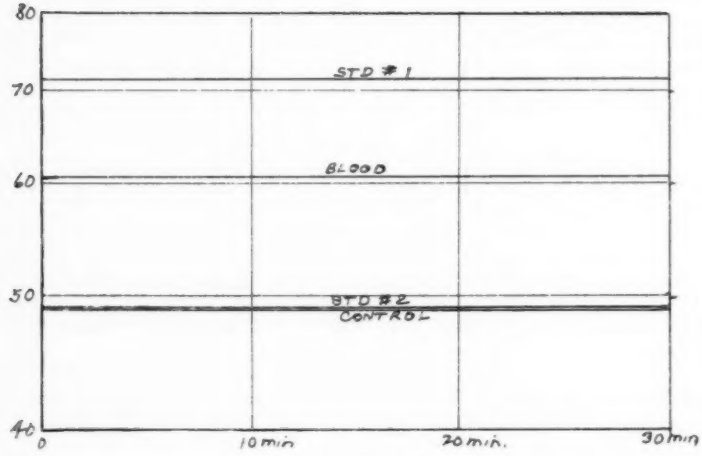
Portions of the filtrate prepared from the undiluted blood were then diluted 1:2 and 1:3 with water. All dilutions and filtrates were prepared in duplicate.

All of these filtrates (a total of 10) were run, along with the

* By about 75 mg.% in the first case, and by nearly 100 mg.% in the second. This refers to the concentration that can be read at a transmittance of 20 or over. The fact that the opposite effect occurs on the dilute end is of little consequence.



420 mu



515 mu

TABLE III

3 standards mentioned previously. Table VI shows the transmittance readings.

The standards were first calculated against each other. At 420 mu, standard #1 against standard #2 read 100 mg.%.

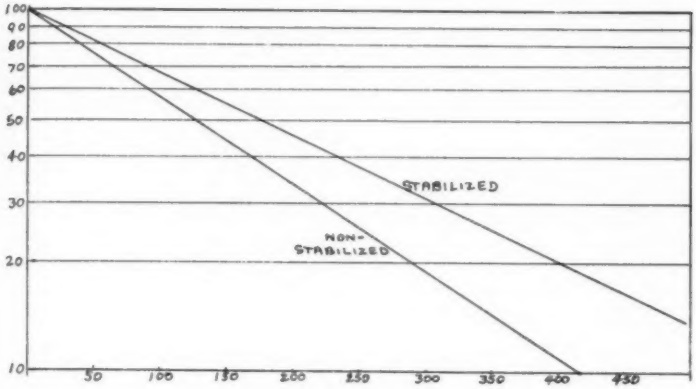


TABLE IV - 515 mu

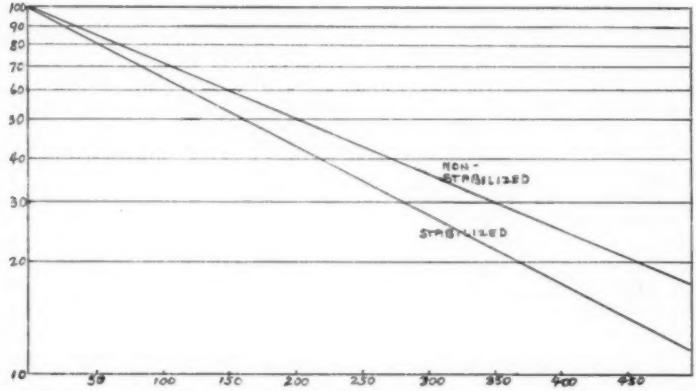


TABLE V - 420 mu

Standard #2 against standard #1 read 194.5 mg.%.

At 515 mu, standard #1 against standard #2 read 98.5 mg.%. Standard #2 against standard #4 read 185 mg.%.

Therefore, the standard #4 readings were definitely off the curve, particularly at 515 mu. According to our curves, which were calculated above 300 and below 100 mg.%, standard #4 should read at a transmittance of 19 at 515 mu, and 17 at 420 mu. In this range, a difference of 1 or 2 transmittance points will give much greater variaton than the same difference toward the center of the scale.

Obviously, from the readings, it is immaterial where the dilution takes place, as long as it is made when necessary.

TABLE VI
Transmittance readings of the undiluted and diluted samples, and standards

	515 mu		420 mu	
	No. 1	No. 2	No. 1	No. 2
Undiluted blood sample	19[1]	19	18	17[3]
Blood sample diluted 1:2	48[2]	48[3]	45	45
Blood sample diluted 1:3	62	61[3]	58	57[1]
Filtrate from undiluted blood sample:				
Diluted 1:2	48[2]	48	44	44
Diluted 1:3	62[3]	62[2]	58[3]	58[1]
Final color diluted 1:2	47[2]	48[2]	44[1]	44[3]
Final color diluted 1:3	62[3]	62[3]	58[3]	58
Standard No. 1 (represents 100 mgm. percent)	66[3]	67[3]	63[3]	64[2]
Standard No. 2 (represents 200 mgm. percent)	44[2]	44[2]	41	41
Standard No. 4 (represents 400 mgm. percent)	17	17[2]	15[3]	16

Table VII shows the actual results obtained by calculating the three blood concentrations—using the average readings—against the three standards. The undiluted blood against standards 1 and 2 gives higher results than the diluted samples read against these same standards. At 515 mu the error is particularly great.

TABLE VII
Showing the calculated results of all blood dilutions against standards 1 and 2, and the undiluted blood samples against standards 1, 2 and 4.

	515 mu	420 mu
Undiluted blood against standard No. 4	376 mg. %	374 mg. %
Undiluted blood against standard No. 1	417 mg. %	388 mg. %
Undiluted blood against standard No. 2	408 mg. %	387 mg. %
Blood diluted 1:2 against standard No. 1	369 mg. %	365 mg. %
Blood diluted 1:2 against standard No. 2	360 mg. %	364 mg. %
Blood diluted 1:3 against standard No. 1	356 mg. %	365 mg. %
Blood diluted 1:3 against standard No. 2	348 mg. %	364 mg. %

3. CUVETTES. Although these discussed above are the main points intended to be made, there is one other detail worthy of mention if greater accuracy is desired.

In doing the readings for this paper, one colorimeter tube was employed throughout each series for making the readings (in addition, of course, to the one containing the reference blank). Most workers have observed the variation that may occur from one tube to another[11]; and in many laboratories, the tubes are checked for optical uniformity before being put to use. If this is done, any variations between tubes would be insignificant. However, in the majority of procedures, it is possible to drain a tube so completely by giving it a sharp shake after natural draining that contamination of one test solution by the fluid

remaining from the last is imperceptible. Of course, this requires a thoroughly clean tube. It is not practical in doing some determinations, such as creatinine, but in glucose determinations, it is both possible and practical. Of course, if a curve is being used, the value of this procedure is lost unless a standard is included in each run.

CURVES VS. STANDARDS. Before closing, a few comments on curves. Although it is generally admitted that, for analytical purposes, curves are not the most accurate means of arriving at an answer[12], their use in a clinical laboratory stems from their practical value; and in many procedures, the results obtained by their use are accurate within reasonable limits. This, of course, assumes that the curves are properly prepared, properly used, and frequently checked. The use of pre-calibrated curves that have not been checked by the laboratory using them is to be deplored under any circumstances.[11, 13]

In some procedures (the BUN by the Gentzkow-Masen method is an example), Beer's law holds true over a relatively short range, so that, if curves are not used, several standards are necessary or the test frequently must be repeated with greater dilutions of the filtrate.[14] Therefore the practicality of a curve is obvious, and the errors involved in its use are not within the scope of this paper. However, this is not true of glucose.[15, 16, 17, 18] These results form a straight line, and Beer's Law holds over the entire range of color within the limits of the transmittance scale. Therefore, one standard—preferably at a level of about 150 mg.%—could serve adequately for any blood sugar value within these limits. Since the preparation of a curve is a tedious and time-consuming procedure at best—and since glucose standards in benzoic acid are easily prepared or purchased, and extremely stable, the advantages of using a curve for this procedure cannot be seen; at least not by the author of this paper. The calculation involved is simply a reversal of that used in visual colorimetry, i.e.:

$$\frac{\text{Density of unknown}}{\text{Density of standard}} \times \text{concentration of standard} = \text{mg.\%}$$

CONCLUSION. As to the most accurate procedure to follow in performing glucose determinations, three general statements could be made:

1. Stabilization of color by re-boiling (or some other means) is essential at most wavelengths, unless very careful timing is used in making the readings.*

2. If color stabilization is not used, it would be desirable for the technologist to observe on his own instrument the color changes that take place at the wavelength he prefers to use, and to establish his reading time thereby.

* Tonks[3] points out that re-boiling also removes the bubbles that sometimes are a source of considerable annoyance by their adherence to the sides of the cuvette.

3. Greatest accuracy is obtained if the unknown is carefully diluted so as to fall in a transmittance range of at least above 20.

In our laboratory and on our instruments, wavelengths of both 420 and 515 mu have been found to be satisfactory. We do not use curves. We use a stabilizing time of 5 minutes. Following this procedure, we are able to obtain very satisfactory and consistent results.

SUMMARY. An attempt has been made to point out some sources of error in the Folin-Wu method for the determination of glucose levels, and to give some practical suggestions for correcting or avoiding them.

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A STUDY OF EFFICIENCY IN THE LABORATORY*

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Efficiency is defined by The Winston Dictionary as "the quality of producing the desired result or the maximum effect with the minimum effort or expense." To apply this definition to the clinical laboratory one might say that the "desired result" is the greatest number of tests per technologist performed with adequate accuracy, in the least time, in the most pleasant and satisfying way for the laboratory, patient and clinician.

The purposes of this article are to outline and illustrate some factors that contribute to efficiency and to encourage a systematic and detailed study of one's laboratory to find where efficiency may be improved. This is admittedly only an introduction to the subject which is so broad that much could be written on any one phase. There needs to be more application in the clinical laboratory of methods used in industry such as time-motion studies. There is need for standardization of laboratory apparatus; perhaps a stamp of approval from an evaluating body.

It is difficult to evaluate the relative importance of the various factors involved. It is evident that good work cannot be done unless the technologist is well trained. Probably the most important factor in efficiency is morale and esprit de corps, although this might be considered a resultant of all factors.

The various factors may be classified as follows:

Physical Factors: Construction; location of the laboratory in the hospital; space; arrangement of rooms and departments; lighting; sound; ventilation; temperature regulation; colors.

Technical Factors: Type and arrangement of equipment and apparatus; maintenance; time-saving devices; worry-saving devices.

Personnel Factors: Training; experience; natural abilities; personal qualifications; morale.

Administration Factors: Laboratory hours, working conditions, salaries, organization (assignments, schedules, selection of technics, batteries and groups of tests, etc.), scientific atmosphere, requests and records, supervision, discipline, conferences, supply. The administration may also be responsible for many of the factors listed previously.

* Based on an exhibit, "Efficiency in the Laboratory," at the meeting of the American Society of Clinical Pathologists, October, 1953. The exhibit received first honorable mention award.

Fig. 1. LABORATORY WAITING ROOM. The entrance to the laboratory is the first contact which the patient, visitors and personnel have with the laboratory and is important in promoting the proper psychological attitude.

Fig. 2. SECRETARY'S DESK. The secretary, seated at the entrance to the laboratory, can take care of many laboratory details. Here she gives information regarding the Blood Bank.

Fig. 3. ELECTRIC TYPEWRITER. Secretary can do in 7 minutes the work requiring 10 minutes on a manually operated typewriter. Typing is easier, less tiring. Makes better looking work, more carbons and better stencils. **Note:** Two telephone extensions help expedite calls to and from laboratory.

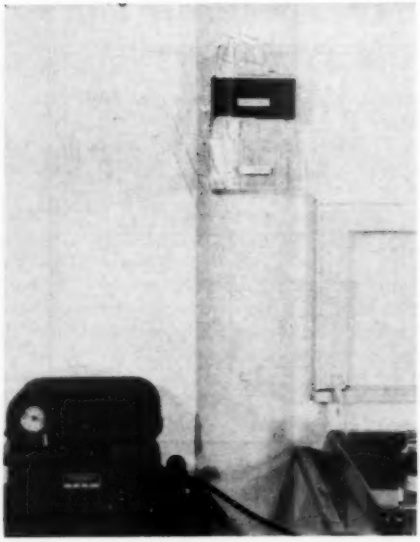

Fig. 4. TIME CLOCK, REQUEST BOX AND SPECIMEN TABLE. A time stamp for request slips and reports settles question of time.

Fig. 5. BLOOD COLLECTING TABLE. Just inside laboratory. Arm rest swings out and may be used for either arm. All necessary materials are within easy reach.

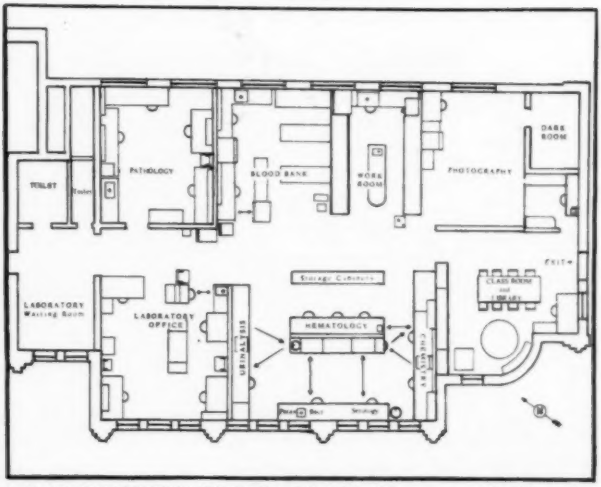

Fig. 6. LABORATORY FLOOR PLAN. "One room," 65′ x 40′ with separation of departments by furniture and partial partitions (except dark room). For 100 to 200 bed hospital.

Fig. 7. FRONT TO BACK ARRANGEMENT. Serology table, with refrigerator immediately behind. Stool can be rolled from front to back to save getting up and down during running of tests. Arrows on Floor Plan, Fig. 6, indicate other places where front to back arrangement is useful, viz., blood collecting table (sink), blood bank (refrigerator), urinalysis (centrifuge), chemistry (centrifuge and prothrombin bath), bacteriology (refrigerator and media cabinet).

THE DEVELOPMENT AND USE OF BCG*

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The importance of BCG vaccination as a means for acquiring specific resistance to the tubercle bacilli has now been recognized in most parts of the world. With the cooperation and guidance of the World Health Organization, there are established BCG centers in varying locales throughout Europe, Asia, Africa, and Australia from where the majority of the vaccine is made up and issued out.

The development of BCG had its initial beginning through the work of Theobald Smith, who was one of the first to realize the fact that varying strains of tubercle bacilli had different degrees of virulence toward humans and animals. After much work and experimental testing, he discovered that the animal strains of tubercle bacilli were less virulent for humans than they were for the animals, and conversely, the human strains had a lower virulence for animals than they did for humans.[1]

Working on this theory, it was Calmette and Guérin who "hit on the idea" that the bovine, or animal, tubercle bacilli might be used for immunizing humans. They attenuated the organism first by sub-culturing on a mixed media of Ox-bile-potato which further reduced the virulence of the bovine bacilli. After cultivating in this manner for five continuous years, the two men noted that the organism which formerly was capable of producing progressive tuberculosis in laboratory animals now failed to do so. They then began immunizing, first laboratory animals, then cattle, and finally monkeys and apes. After numerous experiments, it was definitely established that attenuated organisms did not produce progressive disease in animals, and offered at the same time a protection against natural infection by virulent tubercle bacilli. Such was the beginning of the **B**acillus **C**almette and **G**uérin—BCG.[1]

The characteristic properties of the BCG are very similar to the original bovine tubercle bacillus in terms of morphology and culturing. It takes from seven to ten days to grow on an egg media, and produces coiled colonies of a butter yellow color. Morphologically, the bacillus appears as an acid fast curved rod but differs from the virulent bovine bacillus by not having the characteristic serpentine cord formation. In summary, then, one can say that the BCG is an avirulent, bovine tubercle bacillus which retains the cultural and morphologic characteristics of the bovine mycobacterium.[1]

Up to date, there have been no new basic techniques introduced to improve on the original method of Calmette and Guérin

* Read before First North American Conference of Medical Laboratory Technologists, Quebec, Canada, June 1956.

for preparation of the vaccine. Although the virulence and number of living and dead bacteria vary somewhat from lot to lot, the results of vaccination remain adequately constant so that the lot variations are not really significant.[2]

Because of the short life of the fresh vaccine, the BCG Department of the Pasteur Institute in Paris investigated the possibility of freeze-dried or lyophilized vaccine. When buffered salts and lactose were used as the medium for freeze-dried BCG vaccine, it was found that viability can be maintained for as long as thirty months, and viabil ty in this preparation is claimed by Rosenthal and associates to be equal to that of the fresh liquid preparation for animal resistance and tuberculin conversion.[3] However it is generally felt by others to be inferior to the fresh liquid preparation because of the comparatively low conversion rate and the slower development and shorter duration of the tuberculin sensitivity.[4] Nevertheless, it is still used because of its longer life span, which facilitates the administrat on problems particularly in remote, undeveloped countries, and favorable results have been reported by experienced workers. The WHO has never looked approvingly on the freeze-dried vaccines because of a higher Tuberculin reversion rate, higher mortality of the bacilli, and lower sensitivity.

Through experimental testing, it has been found that the fresh vaccine generally loses over ninety-nine percent of its viability after forty-eight hours of storage; if refrigerated, however, there is little loss of viability in one month. Also it was experimentally found that after refrigeration for one to two weeks, the vaccine gives the same tuberculin conversion as does the fresh vaccine. Dead vaccine gives a very low percent of conversion, while a mixture of dead and live BCG organisms does not alter the high percent conversion rate given by fresh vaccine. In studies done by the International Tuberculosis Campaign, it was found that up to two hundred and fifty-six parts of dead bacilli mixed with just one part live bacilli gave no reduced conversion effect.[3]

As I have previously mentioned, most of the BCG vaccine is now prepared and distributed by the WHO. The United States does not manufacture the vaccine at the present for three main reasons: the vaccine can easily and cheaply be acquired in sufficient needs from Denmark; if this source should break down temporarily, there is always an adequate stock of freeze-dried vaccine available in this country; since they operate on a nonprofit basis, it would be a great cost to build and staff a BCG laboratory.

Thirty-five years ago, in 1921 the first human was vaccinated with BCG which had been transferred 230 consecutive times from the original strain over a period of thirteen years.[1] After

making sure the organism was well attenuated and definitely avirulent, the BCG was administered by the oral route. However, this method of vaccination gave a very low conversion of tuberculin reaction even when up to 10 mg. was given.

Weill-Halle tried the subcutaneous route, and with the injection of only .02-.04 mg. vaccine up to one hundred percent conversion was obtained after nine weeks. However, this method was not entirely satisfactory because of resulting complications, including cold abscesses which persisted for long periods of time.

It was Wallgren, then, who tried intradermal injections. A high degree of conversion resulted, but a high percent of superficial ulcers and occasional abscesses also resulted.

The last method of vaccination to be devised was the scarification method. It was primarily developed for eliminating the complications of abscesses and ulcers which appeared with subcutaneous and intradermal injections.

The principle of scarification is to introduce minute doses of vaccine at multiple sites into the superficial layers of the skin. In this way the patient does not receive the whole dose into one concentrated area. One drop of vaccine is placed on the skin of the forearm and then thirty to thirty-six punctures are made through the vaccine and on into the skin. At first a sewing-needle type instrument was used, but later a more convenient steel disk with thirty-six prongs was devised for faster administration of the vaccine. A week or so after this puncture procedure, small papules develop which remain for two to three weeks and then heal and disappear without gross evidence of any complications or scarring. Up to ninety percent conversion is obtained within the short time of two to four weeks.[1]

Using the intradermal or scarification technique, it is recommended that ten T.U. be administered as a single prevaccination for Tuberculin testing and that the stated two techniques be used in preference to the jelly patch test since the change over from Old Tuberculin to Purified Protein Derivative (PPD).[4]

"Tuberculin sensitivity depends on the presence of tuberculo-protein in the body."[4] It is possible for dead bacilli to produce temporary sensitivity, but for permanent sensitivity the alive tubercle bacilli are needed for continuous stimuli of antibody formation. "In a body which is protected from further infection, an initial small infection of BCG in the form of vaccine will eventually, in the majority of cases, be completely overcome by the phagocytic action of the polymorphs and completely walled off by the proliferation of the fibro-blasts that the lesion becomes sterile and the eventual elimination of all tuberculo-protein will cause a loss of sensitivity to low dilutions of tuberculin."[4] The time for the above procedure to take place varies with the age of the person, virulence of the organism and inherent resistance

of the tissues, and from various records, this loss of tuberclin sensitivity after BCG vaccination can occur in six months or not for five, or even seven years. Therefore reinfection is always necessary.

The degree of immunity cannot be answered in specific terms yet, but there is definite evidence that immunity is conferred after vaccination. The term immunity as used here can be defined as acquired resistance to infection. In many pathologic species of bacteria, the virulent variants possess surface antigens which are essential for virulence, and antibodies against these antigens usually confer specific resistance against these Pathogens. The chemical makeup of the cord formation in the morphology of the virulent strains is not clearly understood, but it is thought to be due in part to an accumulation of a lipid material about the bacilli. It is known that there are certain protein fractions that are responsible for eliciting the tuberculin reaction. These proteins of the bovine and human tubercle bacilli cannot as yet be distinguished from each other chemically, but a differentiation can be made through skin testing and serological tests.

Before speaking specifically of the immunity resulting from BCG vaccinating, I would first like to present experimental evidence that immunity exists against tuberculosis. (1) In the Koch's Phenomenon, when a normal guinea pig is injected with virulent tubercle bacilli intradermally and one to two weeks elapse, it is noticed that the draining lymph nodes are enlarged. The nodule at the site of inoculation becomes necrotic, breaks down and then ulcerates. It is at this point that the tubercle bacilli are dispersed throughout the body, and the guinea pig eventually dies of progressive tuberculosis. On the other hand, if a previously infected guinea pig receives the same dose of virulent organisms, there is an intense redness, swelling, and necrosis within the short time of twenty-four hours. If smears of the necrotic tissue slough are made and examined microscopically, numerous tubercle bacilli are seen. The draining lymph nodes are not grossly enlarged, the culturing is negative, and the lesion at the site of inoculation heals. The guinea pig recovers and does not develop progressive tuberculosis. It can thus be concluded that the previously infected guinea pig had some type of specific resistance which prevented him from getting the progressive disease. (2) In human beings, it has been demonstrated that if a person has had a natural primary infection which has completely healed, as shown by a positive tuberculin and a negative chest film, he will have a higher resistance to tuberculosis reinfection than will another person who has had no primary infection. A positive tuberculin indicates that the

reactor is infected, but does not necessarily indicate he has active tuberculosis.

Age is a very important factor to consider when reading tuberculin tests. If a young child has a positive tuberculin, it usually indicates an active infection which may or may not progress. In adults, however, a positive tuberculin will usually indicate better resistance. In an experiment done on student nurses, it was found that out of 8,000 students who had positive tuberculins only 2.1 percent developed tuberculosis, while out of 4,832 students who were tuberculin negative, up to 5.8 percent developed tuberculosis.[1] (3) It has been demonstrated that only one virulent tubercle bacillus, if placed against intact skin, is capable of producing progressive tuberculosis in guinea pigs. However, if a guinea is first inoculated with avirulent tubercle bacilli, and after waiting for a positive tuberculin a virulent tubercle bacilli is then placed against the infected laboratory animal's intact skin, this guinea pig will have complete protection from acquiring tuberculosis. This again suggests the immunity which the second guinea pig acquired.

It is unanimously agreed by most countries using BCG vaccination that there is a definite reduction in morbidity and mortality of tuberculosis following BCG vaccination.[1] Evidence has shown that BCG tends to prevent primary infection to a large extent, and that when it does occur there is a smaller possibility of the tubercle bacilli spreading throughout the body. However, it must be remembered that BCG vaccination only causes a person to become tuberculin positive, and that humans with a positive tuberculin can still develop tuberculosis. BCG is not a sure prevention against tuberculosis, for, as with most vaccinations, massive reinfection breaks down immunity. The most important factor for preventing tuberculosis is to remove the source of infection, namely the tubercle bacillus itself. There is still a great deal of investigation needed on the tuberculin reaction, for the only interpretation one can now make is that a positive result indicates the development of an allergy to the tuberculoprotein. If this allergy is permanent, then it is highly probable that there are alive tubercle bacilli in the body.

From experiments done with BCG vaccinated mice, Dubos concluded that immunity is related to the number of bacilli present in the tissues.[5] In other words, a larger dose of BCG should produce better immunity. However, it is known that mononuclear phagocytes have the power of suppressing the multiplication of bacilli. Also, it is known that tuberculin sensitivity can only be transferred from animal to animal through mononuclear cells and not passively through the serum of the sensitive animals.[6] Since the number of monocytes in turn is

dependent upon the number of bacilli present this presents a problem that is still not solved.

In using BCG for vaccination, one can conclude that a prevaccination tuberculin test (of recommended ten T.U.) is necessary and that a post vaccination (of ten T.U.) is also very desirable for testing the duration of the tuberculin sensitivity.[4]

A big problem of the future which is yet unsolved is the standardization of BCG vaccines from the many laboratories preparing them. Only vaccines from laboratories which are frequently inspected and approved by an authoritative body should be used.

In summary, BCG has been proven to be harmless, and through its use there may result a reduction of spread within the body if the person is infected or reduce the susceptibility of a noninfected person.

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ABSTRACTS

DETERMINATION OF NEUTRALIZING ANTIBODIES AGAINST MUMPS VIRUS IN HELA CELL CULTURES

Friedrich Deinhardt and Gertrude Henle (University of Pennsylvania—Children's Hospital of Philadelphia) Immun. 77, 40-6 (1956).

The human carcinoma cell tissue culture of the HeLa strain was used in a neutralization test against mumps virus. Basis for the results were the prevention of lysis and degenerative changes in HeLa cells which was caused by the growth of an amniotic strain of mumps virus in the presence of neutralizing antiobdies.

THE BEHAVIOR IN THE COOMBS TEST OF ANTI-A AND ANTI-B PRODUCED BY IMMUNIZATION WITH VARIOUS BLOOD GROUP A AND B SUBSTANCES AND BY HETEROSPECIFIC PREGNANCY

Frederick C. McDuffie and Elvin A. Kabat (Columbia University—Presbyterian Hospital, New York) Immun. 77, 61-71 (1956).

The sera of anti-A and anti-B persons immunized with various blood group substances and by heterospecific pregnancy were examined by the Coombs test in block titration and after neutralization by blood group substances. The reactions fall into two groups: 1, showing enhancement in Coombs titer over the saline agglutination titer and 2, showing no enhancement. The results also indicate that such antibodies cannot be distinguished as being "natural" or immune.

VIABILITY OF ERYTHROCYTES OF ACID CITRATE DEXTROSE BLOOD COLLECTED AND STORED IN AND INFUSED FROM AGED PLASTIC BAGS

Alexander P. Remenchik et al (University of Illinois Department of Medicine and Abbott Laboratories) J. Lab. Clin. Med. 48, 469-70 (1956).

Aged Pliapak blood bags containing ACD solutions were investigated in a series of short and long exposures. The results obtained indicate that prolonged storage (up to 15 months) does not affect the viability indices of erythrocytes: Cr^{51} tagged red cell survival time; immediate postinfusion survival; plasma inorganic phosphorus and osmotic fragility in 0.6 per cent NaCl solution.

A CHALLENGE

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This paper is not a treatise on scientific endeavors; it is not an exposition of medical technology schools; it is not a recruitment article; it does not pretend to give a solution to what is presented as its subject. Most papers do at least that. All the writer wishes to do is to present a problem. It is a genuine one, which demands an honest answer from every true medical technologist.

Considering the demand, technologists are few in number. What profession, percentage-wise, needs more members than theirs? What medical journal does not deplore in each issue the lack of a sufficient number of trained laboratory personnel? What scientific journal does not almost monthly bring out the need of grants, scholarships, etc., to attract students to technology? What atomic interest is not begging for technologists trained in isotope work? What medical school is able to carry out its full quota of research? How many clinical laboratories are able to conduct a twenty-four hour program with skilled professional help? Is the capable internist able to satisfy even his office needs for diagnostic laboratory work? Are state health boards adequately staffed with qualified technologists who can make statistically available results of work which make or break the health of the state? And so on, "ad infinitem."

The problem is not *all* with recruitment—it is not *all* with schools—it is not *all* with public relations—it is not *all* with this or that or the other. The problem, simply, dear technologists, is with *ourselves*. There are not a sufficient number of us who are willing to *teach*, who are willing to *attract* others to our profession by the sharing of knowledge, who are *studious* and alert in "keeping up" (if possible) with technical laboratory medicine, who *present* a professional appearance and ability on every occasion, and who *draw* others by their happiness with a job, satisfyingly performed.

There are many who do—but these many are not *enough!* All are needed to *sell* the medical technology profession. No technologist can be satisfied with having taken care of himself or herself in *this* profession, because technologists are *members* of the *medical* profession—para-medics, or medical associates perhaps we could be called—auxiliaries to the doctor whose work could neither be fulfilled as completely without such service nor could be spread over as great a patient area. And . . .

"The prime object of the medical profession is to render service to humanity" . . .[2] *Service*—service to the patient—service to research—service to our country's needs—service to our community—service to *our profession*. The term and concept of humanity and service could be philosophized throughout the rest of this paper—

[2] Riese, Walther, "Medical Ethics," *Journal Student American Medical Association*, December, 1954, p. 60.

except—that the only part really up for discussion here is *teaching*, is the *sharing* of knowledge with others, is the making of others to feel *wanted* in the profession.

In a personal experience of over twenty years in clinical hospital laboratories as a technologist, and in asking many other laboratory supervisors, it has been noted on numerous occasions the reluctance of most graduate technologists to take an active part in the school's teaching program. The pathologist can no longer do the entire part of the teaching. A school is very fortunate in having pathologists who will lecture as long as an hour a day to medical technology students. Such lectures are best devoted to interpretation of tests rather than to principles and methods in technique. Technologists are taught not only *how* to do tests, but *why* tests are done, significance of results, etc. The pathologists' lecture-province should be far more with the latter of the two mentioned above rather than with the former. The *"how"* of doing is the province of the graduate technologist in the department, and the "how" includes *not only* the technique of tests, *but also how* instruments operate, *how* orders are carried out, *how* the technologists become a vital part of the *medical* team, *how* to be "sentinels of truth" in a hospital. A recent article so called the medical technologist—"sentinel of truth."[3] This same article discussed *how* the work of the medical technologist had vast significance for the social and economic life of the patient. "Slovenly laboratory reports and careless laboratory work always means higher costs to the hospital, to the physician, to the patient. Unnecessary long hospital stays, high costs for nursing service, loss of salaries and wages, worry and anxiety to the relatives and dependents of the patient—all this is frequently traceable to negligence, incompetence or a lack of seriousness in the laboratory.

"It must be admitted that even in the sanctuary of the laboratory there are those, at times, for whom the moral exactions of the laboratory yield to desecration and selfish commercialization. For this reason the school of medical technology must teach ethics along with science; for this reason, the *instructor* in medical technology must possess unselfish love of the truth, if she would realize the high possibilities of her teaching duty."[4]

Reluctance to teaching cannot be attributed entirely to devotedness to the work in the laboratory. Most scientific minds are more eager to *do* rather than to *teach*, and thus many technologists are engaged in this duty of *doing*, and are blind to the duty of *teaching*.

This duty of teaching should be vital to *most* every technologist, if not to *every* single one, who is employed in a laboratory having an approved school. It is true, there should be a clinical instructor,

[3] Schwitalla, Alphonse M., "Duties and Privileges of Medical Technologists," *Hospital Progress*, February, 1956, p. 51.
[4] Ibid.

who is a medical technologist. Many schools do have one. The duty of this instructor is, however, to explain the principles, the techniques, and demonstrations to a *class* of students. It is physically impossible for one instructor to supervise individually and teach each student every technique, and to follow up until efficiency is acquired. The technologist in the various services should assume the responsibility of bring to fruition the material taught to the student in the laboratory classroom. It is in this, we fail—not in having a teacher technologist, but in not having all technologists, teachers! How can this be brought about?

First Things, First

Education of self must come first, and a plan should be made and followed whereby the acquisition of knowledge never comes to a standstill. "The real test of an educated man is his ability to utilize his powers effectively and to good purpose."[5] These active powers can then be directed to teaching.

The teaching which the graduate technologist does, exclusive of that done by the pathologist, by the supervisor, and by the clinical instructor, is less formal than classroom instruction. The instruction often depends upon the interest and readiness of the students for learning, as well as upon the specific procedure. Teaching has educative values, and it takes educational perspective to evaluate the needs of the students. A laboratory teacher, to arouse interest and understanding, must go down to the level of the students' knowledge, then bring them up to a higher and higher level to approach her in technique and in understanding, until the students can in turn discuss intelligently with her, and subsequently on their own, not only the method in question, but also the possible sources of error, and the intricacies which individual tests may present. In other words, ***thinking a problem through*** becomes a part of the student's second nature because of the explanation, the interest, and the patience of the graduate technologist. Students do not learn, or rather should not, by just observing, without actually performing the test. It is not unknown that some graduate technologists, rather than being helpful, makes the student trainee very uncomfortable and insecure while in their particular section of the laboratory. The graduate should be friendly, alert, active and precise on her job. Efficiency is contagious and good actions speak louder than words.

Technologists should be sure, however, there is a "meeting of minds" in her explanations to the student. A teacher talking "over the head" of a student is ineffective, to say the least. When the performance of a student is worthy of praise, then

[5] Perrodin, Cecilia, "Supervision of Nursing Service Personnel," The MacMillan Company, New York, 1954, p. 24.

give praise constructively. "Praise the accomplishment more than the person; after all, he knows it is his work. Criticize constructively, too, discussing not the individual, but an aspect of his work."[6]

Teaching Principles

An excellent analagous outline between teaching principles and supervisory principles is brought out in one of the early chapters of "Supervision of Nursing Service Personnel.[7] This outline clarifies the need of the transfer of the knowledge of teaching principles to students, giving the graduate technologist a realization of the value of teaching. "Education has many definitions and interpretations. Derived from the Latin e-duco, meaning "I lead out," education is best defined as the leading out of all of a student's potentialities and assisting him to develop them and utilize them to the maximum of his ability."[8]

Good teaching never loses sight of the fact that the hospital laboratory differs from ordinary science laboratories in that there can be no trial and error tests. The student should be impressed, too, with the realization that techniques vary from one laboratory to another, and that what is taught in a local situation is the local method, but not the *only* good method.

Good teaching utilizes standards of checking and grading students which evaluate not only the student's personality but also her skills, her ability to use knowledge in practice, and appreciations essential to her development. Finally, good teaching fosters an inquiring attitude of mind, initiative, and self-direction. Remember, fellow technologists, a good teacher is not made overnight. Study, experience, good will is as aptitude. Your profession as a medical technologist presupposes, always, an *educated* person.

Student Opinion

The author, in thinking about this paper, considered it would be well to have the six students enrolled under her supervision, express themselves as to what they wanted and expected to find in the graduate technologist in charge of each department. While, understandably many of these students comments were repetitions of one another, each student had some different comment to offer on the teaching angle. The following are a few excerpts from their papers.

"When an atmosphere of happiness prevails, work is taught better, faster, and more accurately."

"Present all important facts and techniques in an orderly fashion. The slow student's schedule can be limited to the essen-

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[6] Norman, Edward, "Techniques of Supervision," *Journal of the American Dietetic Association*, October, 1955, pp. 997-1001.
[7] Perrodin, Cecilia, "Supervision of Nursing Service Personnel." The MacMillan Company, New York, 1954, pp. 19-23.
[8] Ibid.

tials while the fast student's schedule can be expanded according to his ability."

"The most valuable quality of the graduate is self-respect. The graduate must always remember that he sets the example for the students. If he does not have any pride in his work, he does poor work. If the graduate is sloppy in appearance, he cannot command any respect from students or patients. Without respect the graduate loses the effectiveness of all his other qualities."

"A technologist must always be ready to help a student. This readiness must be available from 8 a.m. to 5 p.m.—not just when she feels like it."

"A technologist must be willing to help. Some seem to think of the students only as 'peons' with little knowledge and common sense. Does she ever stop to realize someone had to teach her?"

"We might not be the best class of student technologists to be found, but we are deserving of the readiness, willingness, and ability of the registered technologists."

"I expect from the technologists a real desire to help us and share as much of their knowledge with us as possible. I expect them to have the knowledge and ability to make a good technologist out of me."

"I don't expect my instructors to know the answer to everything, but I expect them to be able to tell me where to find the answer."

"I realize you only get out of something exactly what you put into it. If every student realized this, there would be fewer headaches for the instructor-technologists."

"I would prefer to be considered irgnorant of any procedural technique than to be considered someone who knows all about it before I am taught. I want my teacher technologist to start me at the bottom, making sure I know the fundamentals."

"The technologists know what we are expected to do and what valuable information we can use. They should make sure that we know what we are expected to do while in any one department of the laboratory."

"The student should be given an opportunity to become proficient in performing the various tests, and not aim solely at performing the specified number of tests."

"You have to learn medical technology so that you will never forget. This requires a different kind of teaching and a different kind of learning than most of us have had in college."

"In order for me to have the self confidence I feel necessary, I must know every principle for every procedure I do."

"I am not looking for self-pity when I ask for patience in an

instructor. I feel that a student can do better work if she feels like someone understands."

"Working with people's lives can make even a small mistake a serious one. It is for this reason that I expect a great deal from my instructors and for this reason I want my instructors to expect a great deal from me."

Such, dear technologists, are the thoughts of students who are about half way through their hospital training. Do you see why we must teach, or learn how to teach, to satisfy these eager minds entrusting themselves to us? The responsibility is everyone's—not just the head technologists. Only by active participation are we being true promotors in our profession.

Conclusion

The phenomenol increase in the need for additional laboratory personnel in the past decade, and the even more phenomenol potential need for the next decade, challenges the loyal medical technologist. In adding to his own professional growth year after year, he must be mindful also of the help he needs to give to those starting in the profession.

Progress in the clinical laboratory will continue as other endeavors forge ahead i.e. radioisotopes, electrophoresis, chromatography, fractionating proteins, and all the other new names appearing in the laboratory vocabulary. No one can afford to be lethargic, complacent, and averse to change. No one can afford to let her profession down by refusing to teach. The challenge, it seems, is all too obvious! Can we meet it? What is your answer to this problem?

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ABSTRACT

PROCEDURE FOR CLINICAL EVALUATION OF RENAL FUNCTION

Lawrence G. Raisz et al (Veterans Administration Hospital—Boston University, Boston) J.A.M.A. 162, 266-8 (1956).

In an evaluation of the renal status of patients it is desirable to include one test that indicates the magnitude of the glomerular filtration rate and another that serves as a measure of renal tubular function. This is of particular value with those patients whose plasma concentrations of nonprotein nitrogen or urea may be slightly elevated while creatinine clearance values may have fallen as low as 50 or 25 per cent of normal. A single method is given for measurement of endogenous creatinine clearance and phenolsulfonphthalein excretion during a water diuresis as a useful adjunct to the clinical evaluation of renal function.

STANDARDS, CONTROL CHARTS, AND RELIABILITY IN THE DETERMINATION OF SERUM PROTEIN

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A recent survey of twenty-nine clinical laboratories in Northern California indicated twelve different methods, or published modifications, of protein determination were in common use. The reported methods were tabulated[1] as follows: 10 laboratories utilized the biuret reaction (8 modifications); 9 the reaction of tyrosine with a phenol reagent; 6 utilized Nesslerization; 3 the Kjeldahl and one a turbidimetric method. The accuracy and reliability as well as the difficulty of performance of these several methods is quite variable. The method of determination of total nitrogen initiated by Kjeldahl[2] in 1883 still remains as the reference method for quantitative protein estimation. However, that relatively few laboratories utilize the Kjeldahl technic points out the fact that despite its acknowledged dependability, usefulness is restricted, since the technic is relatively time consuming and difficult. Of the color reactions introduced to replace the Kjeldahl, the two most frequently used are that of tyrosine with a phenol reagent[3] and that of peptide linkages with a copper complex. Neither has been entirely satisfactory in the opinion of all investigators.[4,5]

Of the colorimetric methods, those based on the biuret reaction appear to require the fewest number of reagents and manipulations; however, a solution of known protein content is required as a standard. It was the intent of the study to collect data concerning the stability of various protein preparations which might serve as standards for the biuret method, and to evaluate the reproducibility, reliability, and accuracy of this method of protein determination in comparison with the Kjeldahl technic for which it is often substituted.

Methods and Materials

The biuret method described by Weichselbaum[6] was used. Although this method is less sensitive than some other biuret modifications, the simple protocol* provided opportunity for careful control of technic, and the reported stability of the reagents was a desirable feature. The Weichselbaum "sensitive" reagent (1.5% $CuSO_4$ in 0.2N NaOH) was used in all determinations. Following incubation at 37° C the colorized test mixtures were cooled to room temperature (20-23° C) before determining the optical density in either the Beckman DU or Coleman Jr. spectrophotometer. No change in optical density

* Addition of 0.1 ml. serum to 4.9 ml. normal saline. To this serum dilution is added 5.0 ml. of biuret reagent. The mixture is incubated 30 minutes at 37° C and its optical density determined.

† Supported in part by Faculty Research Grants, University of California.

was noted for test mixtures varied through this 3 degree range.

As a reference method, the micro Kjeldahl analysis was carried out according to the technic described by Kabat and Meyer,[7] but was not maintained as a routine (daily) analytical tool through the two-year period. Various checks on accuracy were employed throughout the study, however, i.e., amino acid and ammonium sulfate recovery; agreement with Kjeldahl values from an official food and drug laboratory and from a licensed biological firm.

To determine whether the biuret method could yield reproducible results, multiple tests were run on a single pool of serum. The accuracy of the method was estimated by comparison with Kjeldahl values obtained on the same serum pool. The serum was divided into 5 ml. aliquots, placed into 3 dram rubber stoppered vials and kept at —10° C. The specimen for any one day's determinations was thawed, mixed by inversion; the required amount of serum was removed and the remainder refrozen at —10° C. Twenty-one determinations were made by the Weichselbaum technic on the morning of the first day; twenty-five in the afternoon, and nine on the second day. The Kjeldahl determinations were carried out, eight to ten per day, over a period of one month. As an additional index of the reproducibility of the biuret method, duplicate tests were made on 86 different human sera.

In order to evaluate the several storage methods for protein standards, serum, plasma and albumin were stored over a two-year period as follows:

(1) **Plasma.** (a) Diluted. Dessicated, irradiated, pooled human plasma was reconstituted to its original volume with sterile distilled water. Separate 20 ml. portions of the reconstituted plasma were diluted 1 to 50 with normal saline (0.85% NaCl) and 1 to 50 with 15% NaCl solution. The portions of diluted plasma were rebottled as 25 ml. aliquots in 30 ml. soft glass bottles, closed with cork stoppers and stored at 2 and —10°C as specimens I through IV*.

(b) Undiluted. Fresh, pooled, normal, human plasma obtained from blood collected in dry potassium oxalate was stored as specimen V in 0.5 ml. portions in the frozen state without a preservative in Pyrex test tubes (13 x 100 mm.) closed with cork stoppers.

* Dry NaCl was added directly to another portion of the reconstituted plasma in the amount of 15 g. NaCl/100 ml. plasma. This preparation yielded a precipitate which, after washing, gave a positive xanthroproteic reaction. The mixture was excluded from a further consideration. Addition of 30% urea (aq.) plus thymol[8] to the reconstituted plasma resulted in an optically clear solution which may prove effective as a standard for the biuret method; however, it was noted that when stored at —10° C for three years, this preparation solidified and would not become liquid at any temperature below its coagulation point. The presence of the relatively large amount of urea however tended to obscure the significance of the Kjeldahl values of protein nitrogen and this preparation was excluded from further determinations.

(2) **Serum.** Pooled, normal, human serum with dry NaCl added in the amount of 15 g./100 ml. serum was placed in 2 ml. aliquots, in 2.5 ml. vials closed with cork stoppers and stored at 2 and —10°C as specimens VI and VII.

(3) **Albumin****.** Albumin, salt poor, which had been commercially prepared from human plasma for intravenous administration[8] and shown by electrophoresis to be 99+% pure. The sterile albumin solutions, obtained in 25 ml. amounts in vaccine stoppered vials were kept at 2°C. Aliquots (2-3 ml.) were removed with a sterile hypodermic syringe and transferred to 10 ml. glass vials which were closed with tapered rubber stoppers. Some aliquots were kept at 2°C, others at —10°C as specimens VIII through XI. When appropriate, the preceding solutions were sterilized by Seitz filtration and dispensed into sterile containers; thereafter no particular precautions were maintained to preserve sterility. After each protein solution had been prepared, the protein concentration of a representative sample was determined by the Kjeldahl and the biuret method. After storage for varying lengths of time at the selected temperature levels, samples were removed for protein analysis. In the case of the salted serum samples, the contents of any one vial were tested on one day only, and the remainder discarded. The plasma and albumin samples were tested repeatedly from the same vial.

To determine whether there was an appreciable rate of evaporation from containers used in this study, repeated weighings of some specimens were made. The weights were recorded for both stoppered and unstoppered vials containing 2 ml. or less of the pooled human serum.

As a determination of whether the protein concentration would change with appreciable water loss, serum was placed into several weighed shell vials, 1 x 3 cm. The vials were left unstoppered at room temperature (75-80°F), and at selected intervals the weight was determined by weighing again and the protein content measured by the biuret determination.

Using two concentrations of the human albumin selected as a standard, transmission-concentration (T-C) curves were prepared for the Beckman DU on each of 19 days during an eight-week period (16 April-15 June) and on each of 10 days for the Coleman Jr. (19 May-15 June).

To determine whether the biuret reagent changed with time, or type of storage container, two lots of reagent were prepared one month apart. Each lot was separated into soda glass and pyrex glass containers and kept at room temperature. On the third month, see Figure 1 and 2 (15 June, 1953), both lots were tested, with and without filtering the reagent before use, by

** Cutter Laboratories, Berkeley, California. Each lot obtainable with individual Kjeldahl value as Normal Serum Albumin (Human) Salt-Poor, U.S.P.

colorizing aliquots of the same standard albumin as was used above for T-C curves. These same lots of reagent were tested again 9 months later (10 and 11 months respectively after preparation) utilizing the same albumin standard.

Results

Reproducibility and Accuracy of the Biuret Method: The protein content of the pooled serum as determined by multiple determinations was:

Weichselbaum:

1st. a.m. Series 21 tests, mean value 7.20g.%, s.d. 0.1g. (range 7.1-7.4g.) 2 s.d. = ± 2.8%

1st p.m. Series 25 tests, mean value 7.71g.%, s.d. 0.152g. (range 7.5-8.1g.) 2 s.d. = ± 3.9%

2nd a.m. Series 9 tests, mean value 7.56g.%, s.d. 0.141g. (range 7.3-7.7g.) 2 s.d. = ± 3.7%

Kjeldahl: 22 tests, mean value (corrected for NPN) 7.52g.%, s.d. 0.083g. (range 7.2-7.6g.) 2 s.d. = ± 2.1%

The reproducibility may be computed as the variance of each series and method and expressed in terms of s.d. (σ) or range. The mean value of the 55 biuret determinations (7.49g./100 ml.) differs from the Kjeldahl mean value by 0.03g. (a difference which is less than one standard deviation for the Kjeldahl determinations) and may be taken as a measure of the accuracy of the biuret method.

It was found for the paired Weichselbaum determinations on each of 86 different sera, read on the Beckman DU, that the reproducibility measured as the mean difference between the total protein value for any one serum and its duplicate was 0.17g. with a range of 0.0 to 0.5g. in 85 of the 86 paired determinations, and one pair differing by 1.2g.

Prolonged Storage. The values obtained for the protein concentration of the stored samples, I through XI, are presented in Table 1. Each value is the mean of two or more determinations and is listed as a percentage of the original protein concentration. Allowing for method variance according to the 2 standard deviation limits presented for a standard control chart (v.i., p. 370) the values for protein concentration as determined by the biuret method, increased for all samples except V and X. The increase in concentration is not as great for frozen specimens (I vs. II, VI vs. VII, X vs. VIII, IX and XI).

Evaporation. The data in Table 2 indicate a rapid loss of weight (about 2 mg./hr.) within the first hour of unstoppering a vial containing a pooled serum specimen. The rate of loss reached a relatively stable value at 2 hours (about 1 mg./hr.). The rate increased again to about 2 mg./hr. after the vial

TABLE 1

Protein Concentration of Stored Specimens of Serum, Plasma and Albumin Concentration as Percent of Original Value as Determined by Biuret and Kjeldahl Analysis

Storage Time in Months

Sample		0	1	8	10	11	20	22	24	30	Reliability of Biuret Method†
I Plasma, 1-50 in 0.85% NaCl 2°C	K*	100	92.5	97.2	93.6	100	97.4	....	.....	...	.....
	B	...	.....	96.0	.....	112.3	.....	113	.....	...	± 9%
II Plasma, 1-50 in 0.85% NaCl —10°	K	...	.....	.....	.....	.....	.....	.....	.....	...	.....
	B	...	.....	.....	.....	.....	.....	.....	.....	111	± 9%
III Plasma, 1-50 in 15% NaCl 2°C	K	100	98.4	102.5	102.8	.....	94.0	96.1	108.1	...	.....
	B	...	.....	96.0	.....	112.3	.....	.....	.....	...	± 9%
IV Plasma, undiluted base of the above —10°C	K	...	.....	.....	.....	.....	.....	.....	.....	...	.....
	B	...	.....	.....	.....	.....	.....	.....	114	...	± 9%
V Plasma, pure —10°C	K	100	104.4	102.9	.....	.....	.....	103	.....	...	.....
	B	...	.....	90.0	.....	115	.....	108	.....	...	± 8%
VI Serum, with 15g. NaCl per 100ml 2°C	K	100	104	105.6	.....	111	114	112.5	.....	...	.....
	B	...	.....	83.0	.....	124	.....	118	.....	...	±10%
VII Serum, with 15g. NaCl per 100ml —10°C	K	...	.....	.....	.....	.....	.....	.....	.....	...	.....
	B	...	.....	.....	.....	.....	.....	.....	.....	116	± 9%
VIII Albumin 2°C	K	100	.....	.....	104.2	100.3	93.3	111.9	.....	...	.....
	B	...	.....	.....	.....	.....	.....	113	.....	...	± 7%
IX Albumin 2°C	K	100	.....	.....	100.7	101.8	94.8	113.9	.....	...	.....
	B	...	.....	.....	.....	.....	.....	118	.....	...	± 7%
X Albumin —10°C	K	100	.....	.....	101.8	97.4	.....	99.1	.....	...	.....
	B	...	.....	.....	.....	.....	.....	100	.....	...	± 7%
XI Albumin 2°C	K	100	.....	.....	100.4	98.9	96.9	115.1	.....	...	.....
	B	...	.....	.....	.....	.....	.....	111	.....	...	± 7%

*Method of determination: Kjeldahl or Biuret.
†Percentages given for calculation of limits of variation of Biuret Method as based on two standard deviations of the mean standard curve.

(straight sides, flat bottom, without lip, 1 x 3 cm.) had been tilted and rotated so as to cause the serum to "wet" the walls of the vial, i.e., to increase the surface area of the specimen. This weight loss occurred at room temperature within a draft-free balance case when the serum occupied the lower 1 cm. of the vial. The rate of evaporation was greater from a similar vial, the lower 2 cm. of which were occupied by the serum, i.e., the surface of the serum was closer to the lip of the vial. In this case the loss amounted to 5 mg./hr.

From similar vials, also at room temperature, but with cork stopper firmly in place, the losses amounted to 0.85 and 1.05 mg./day. Throughout the observation of the stoppered vials, it was noted that the portions of the cork stoppers which were

in contact with the walls of the vial were always moist even though the vials had never been inverted nor the serum allowed to wet the cork or the upper portion of the vial. These vials were of the same type as were used to store samples VI and VII.

TABLE 2

Weight of Stoppered and Unstoppered Vials of Serum and Rate of Loss During Storage at Room Temperature

	STOPPERED		UNSTOPPERED		
	Weight (g.) Vial, Stopper and Serum		Weight (g.) Vial and Serum		
Days	Sample 1 1 ml. Serum	Sample 2 2 ml. Serum	Hours	Sample 1 1 ml. Serum	Sample 2 2 ml. Serum
0	2.8814	4.3861	0	2.6435	.....
1	2.8809	4.3820	.5	2.6414	4.1039
4	.....	4.3778	1	2.6401	4.0989
6	2.8786	.....	1.5	2.6391	.....
8	2.8758	.....	2	2.6382	4.0971
14	.....	4.3713	2.5	2.6375	4.0954
18	2.8670	.....	3	2.6366	.....
28	2.8576	.....	3.5	2.6358	.....
47	2.8415	.....	...	.....	.....
Loss	0.85 mg./day	1.05 mg./day		2.2 mg./hr.	5.7 mg./hr.

The degree to which the protein concentration, as determined by biuret analysis, agrees with the calculated protein value based on known weight loss attributed to evaporation of water of specific gravity of 1.000 is shown in Table 3. The specific gravity of the original solution was obtained by applying the formula, Protein Concentration = (sp. gr. —1.0073) 347.9, given in Gradwohl.[9] The material in the vial was not measured volumetrically but its volume calculated by known weight loss, i.e., that attributed to evaporation (sp. gr. 1.000) and that due to removal for analysis of a known weight of solution (converted to volume by dividing by the specific gravity calculated after evaporation). No corrections were made for volume occupied by the solutes. The poor agreement among the high protein values may indicate the upper limit of the biuret method, analytical error or cumulative error in the calculated value. The latter seems unlikely since the highest calculated value was used in the calculation of the protein content after replacing the water lost by evaporation.

Control Chart. The instrument readings obtained from the Beckman DU and from the Coleman Jr. spectrophotometers for 4.28 and 8.56 g./100 ml. human albumin standards (Sample X) are given in Figure 1 and 2 respectively. The mean and 2 standard deviation values of %T shown on Figure 1 were calculated from data collected over the entire eight-week period (16 April-15 June). For the Coleman (Figure 2) the mean and 2 standard

TABLE 3

Weight and Protein Concentration of Serum Samples Stored in Unstoppered Vials at Room Temperature

Sample	Time (Hours)	Weight (g.) of Serum		Protein Concentration in g. per 100 ml		
		Before Removal for Periodic Test	After Periodic Test	Calculated	Biuret	Difference
B	0	.....	1.2194	....	7.7	....
	28	1.0832	.9712	9.11	9.55	.44
	48	.8967	.7842	10.12	9.72	.40
	120	.4220	.3125	21.53	18.50	3.02
C	0	.....	.8266	....	7.7	....
	28	.7356	.6239	8.97	9.40	.43
	48	.5710	.4612	12.0	9.78	1.22
	120	.1988	.0869	32.84	24.0	8.84
D	0	.....	.8245	....	7.7	....
	28	.7086	.5926	9.39	9.50	.11
	48	.5277	.4173	10.96	10.52	.49
	120	.0912	.0890	68.29	*	....
E	0	.....	.9555	....	7.7	....
	28	.8091	.6992	9.60	9.63	.03
	48	.6482	.5350	10.57	10.10	.47
	120	.2777	.1684	23.41	20.5	2.91
		Water added to replace calculated evaporative loss				
B	....	.7869	.....	8.20	7.60	.60
C	....	.4893	.....	5.37	5.70	.33
D	....	.5884	.....	7.62	7.58	.04
E	....	.7109	.....	4.22	4.90	.68

*Too thick to pipette.

deviation values of %T were calculated from data collected during the first month only (19 May-15 June).

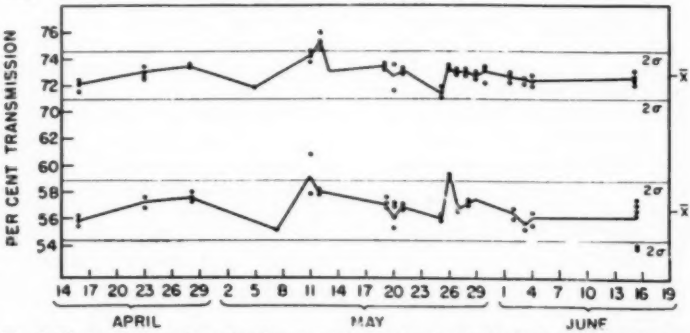

Fig. 1. Photometer readings for biuret total protein standards. Beckman DU, 555 mu. Individual readings as plotted. Curves connect calculated daily mean values. Upper curve for 4.28 g./100 ml., lower curve for 8.56 g./100 ml. standard. The per cent transmission values are shown in linear scale for simplicity and their logarithmic relationship is not depicted.

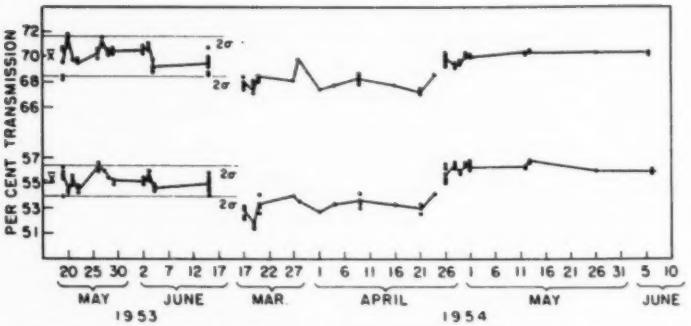

Fig. 2. Photometer readings for biuret total protein standards. Coleman, Jr., 555 mu. Individual readings as plotted. Curves connect calculated daily mean values. Upper curve for 4.28 g./100 ml. standard, lower for 8.56 g./100 ml. standard. New reagent used starting 26 April, 1954.

Reagent Stability. When the reagents were examined within 1 and 2 months of reagent preparation, the instrument readings obtained at each protein level tested, irrespective of age or manner in which the reagents were stored, were all within the range obtained in preparing the mean curve using only one reagent. When examined 10 and 11 months after preparation, similar agreement in the range of readings was obtained. However, the absolute values of the blank and standard readings were always less than those obtained 9 months earlier (Figure 2, May-June, 1953, vs. March-April, 1954). However, a second biuret reagent prepared later gave, in May 1954, essentially the same readings as those of the first reagent in May 1953 (Figure 2). The aged reagent decrease averaged 1.9%T for the blank, 1.6%T for 4.28 g., and 1.4%T at 8.56 g. The data are insufficient for test of statistical significance, but the decrease recorded for soda glass stored reagents averaged 0.5%T more than for pyrex. Likewise, filtering the reagent before use usually resulted in a decrease 0.5-0.6%T more than if the reagents were not filtered.

In view of the reagent instability reported here and by Henry and Segalove,[10] the use of an artificial[11] standard which does not require addition of the biuret reagent (which must be used in the unknown) conceivably may lead to additional uncertainties. The obvious advantage with a permanent artificial standard, if maintained in sealed cuvettes, of avoidance both of evaporation and error from cuvette variation, is counterbalanced (1) by the need of identical slope and magnitude of the artificial standard T-C curve and the TC curve of biuret reagent mixed with serum of known protein concentration (or a mathematical factor to compensate for lack of such agreement) and (2) by the periodic

need of a standard serum to confirm reagent stability.

Discussion

Wokes and Still[12] and Kibrick[13] reported an instability of stored serum proteins as determined both by biuret and tyrosine methods. These investigators observed that the intensity of the biuret color may be markedly increased in aged samples of serum. The disconcerting observation that Henry and Segalove **(loc, cit)** " . . . have seen a refrigerated biuret reagent deteriorate within 48 hours to the extent that standards read 30% below what they should have read" tends to emphasize the concern of others that biuret reagents are not "stable indefinitely" under practical conditions. Thus the combination of the two possibilities, variation in the standard serum, and reagent instability, may lead to serious difficulties in the absence of other methods (eg., Kjeldahl analysis) for periodic standardization.

From the data in Table 1 it seems likely that the increase in protein concentration as determined by biuret reaction cannot be entirely ascribed to the alteration of biuret active linkages or other alterations of the protein, or to interfering substances, in view of the parallel increase in total nitrogen as demonstrated by the Kjeldahl analysis. If such increase were to be accounted for, in part at least, by evaporation, then it is due to a surface phenomenon and, other factors such as surface area of the liquid, cross sectional area of the bottle opening, etc., being equal, the weight loss should be absolute and the consequent percentage effect on protein concentration should become greater the smaller the volume of the sample. This indeed was the case as noted with the samples stored. Albumin samples VIII, IX, and XI, stored at 2°C showed a marked increase during the 21st to 22d month while sample X, similarly stored, did not. Sample X contained 2-3 ml. albumin in a 15 ml. rubber stoppered vial, whereas each of the other three samples, in similar containers, was less than 1 ml. in volume. By the 23d month, (one month after the last Kjeldahl analysis) sample XI had evaporated practically to dryness and in VIII and IX a marked increase in viscosity was demonstrated. Each of these samples was in a glass vial and closed with a rubber stopper driven home with sufficient force so as to make one believe that closure was as complete as could be expected with this method. For the plasma specimen (1) diluted 1-50 with 15% NaCl bottled in 25 ml. amounts and stored at 2°C, no consistent change was demonstrated until the specimen had been almost used, i.e., only 3-4 ml. remained, after which the values for protein concentration increased markedly. On the other hand, the plasma (Sample V) which was kept at —10°C (in 0.5 ml. amounts) showed only a 3% increase in 2 years and aliquots of human albumin (Sample X), which were kept frozen whenever rebottled in 2 or 3 ml.

amounts, increased less than 2% in protein concentration over a storage period of 18 months.

From the data given here, it appears possible that the majority of such increases may be explained by postulating the evaporation of water from the protein solution, in that (1) biuret increase was always accompanied by a total nitrogen increase, (2) protein concentration as determined by the biuret analysis closely parallels the concentration calculated from known weight loss even though the sera were left in open containers at room temperature (70-80°F) and became grossly contaminated. If other processes also were involved then the combined increase (evaporation plus bacterial action, etc.) should have been greater than that due to evaporation alone and greater to the extent noted by Wokes and Still and by Kibrick.

In accord with the trend toward micro-procedures, the biuret method seems admirably suited as a companion tool to present day paper electrophoresis technics since the Weichselbaum biuret method can yield protein values which are accurate and are regularly reproducible within ±4% for determinations performed consecutively on the same day and since frozen sera can be a reliable protein standard. The chief difficulty with the technic lies in the day to day variance in spectrophotometer readings. As an investigation of such variance the control chart method was applied (Henry and Segalove, **loc. cit,** and others).

This day to day variance (Figure 1) in the apparent protein concentration of the same serum sample may serve as a measure of the reliability of the method, or the accuracy over a period of time. The 2 standard deviation values calculated for these standards for 3 months (reliability) indicate that 95% of the values would fall within ± 0.6 g. of the mean (or true) concentration—which includes all the daily mean values. Such a calculation of reliability defines a confidence interval which is more realistic than that obtained from the range of daily replicate values (reproducibility) which here did not exceed ± 0.15 g. It is, then the reliability of a method which affords the clinician the best measure of the confidence to be placed in a single report.

Summary

It has been shown that plasma, serum, or commercially obtainable albumin for human injection may be maintained as a satisfactory standard preparation for use with the biuret method of protein determination for periods up to two years. The presence of preservatives (or bacteria in the absence of preservatives) is of less importance than control of evaporation. Evaporation may take place at a measurable rate from glass vials closed with tapering rubber or cork stoppers. Increase in the values of the protein concentration of stored standards proceeds more slowly at —10°C than at 2°C.

Weichselbaum sensitive biuret reagent may change after 3-9 months storage at room temperature. The change is somewhat less for reagents stored in pyrex glassware than in soda glass. The change is magnified by filtering the reagent before use.

The accuracy of the biuret method was found to be high in a series of 55 determinations on a serum pool, the mean value differing from the corresponding Kjeldahl value (7.52 g./100 ml.) by 0.03 g. This accuracy combined with the small volume of sample required for the technic make the biuret method a satisfactory companion tool for paper electrophoresis of serum proteins. The reliability, that is the day to day variation in the biuret reaction as measured by the range of spectrophotometric readings obtained for a given protein specimen is in general larger than the reproducibility, that is the variation in multiple determinations made on the same day. The biuret method was found to be reproducible to ± 0.15 g.; the reliability based on the two standard deviation level calculated from data collected over a two-month period, was ± 0.6g. at any protein concentration tested. The estimation of the protein concentration of a serum sample is best interpreted in terms of the reliability rather than the reproducibility of the method.

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APTITUDE TESTING FOR POTENTIAL STUDENTS IN MEDICAL TECHNOLOGY*

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In the last twenty years laboratory medicine has made great progress, and with it more exacting work is demanded from those who have chosen Medical Technology as their profession. We need people who have method, self-sacrifice, watchful activity, love of their work, devotion to duty, courage and sympathy. These qualities will give us technologists who have an intellectual interest in their work, a hearty interest in the patient, and a technical interest in the patient's cure.

Every creature has a special work in life to do. If we can be of any assistance in helping an individual find his or her chosen profession we have done a great charity. On this will rest the health and happiness of the individual.

Several years ago after organizing an Approved School of Medical Technology in a two hundred bed hospital, a problem presented itself, "How can students be evaluated before being accepted into our school?"

The majority of students had their college degrees, others had the equivalent of three years and about ten percent had the minimum requirements of the Registry of two years. A small percentage of the students were lacking the scholastic standing one would expect of an individual with higher education.

I was evaluating the students by the ordinary means of transcript, letters of recommendation, correspondence and interviews whenever possible. Several of the students had to be eliminated after a period of time due to scholastic standing, even though their letters of recommendation were beyond reproach.

I asked myself if it was not possible to protect the profession, the individual student, and myself by an elimination process through the administration of a battery of tests before the entry of the student into the school. I was convinced of the need for a sounder method of identifying intellectual ability and one which would include the evaluation of feeling, thinking and acting.

Personality is not something separate and apart from ability but includes them. When a teacher has evidence showing the student's intellectual capacity and skill, knowledge of a student's characteristic modes of response in a variety of situations which affect him as an individual, or as a member of a group, he can use this more complete picture to guide him to better personal and social adjustment.

Realizing that I was a member of a very honorable profession

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and that I had a moral obligation to society to admit only students who gave me reasonable assurance that they would benefit humanity, I carried my problem to a testing specialist.

I made an appointment with a Professor who headed a department of Testing and Counseling in one of our State Universities. He was kind, courteous, enthusiastic, and most cooperative. He explained to me that Aptitude Tests were designed to measure the fitness of the student for performing a specific activity or work. All schools should have positive proof in selecting students. He explained how some schools treated this in a subjective manner, for instance, if applicants seemed nice, willing, looked intelligent, appeared well and strong at an interview, they were accepted without further delay.

The Professor further explained to me that there were two effective ways of determining Aptitude: one, on the job training, and the other, by administering a Standard Test. The first method had evident disadvantages, namely, discouragement, needless expense, and embarrassment on the part of the student, and costliness to the institution. The second way provided definite objective and fairly dependable information about the traits and capabilities of the individual. The predictive value of this type of test, he said, is not high, in the sense that it is an infallible criterion for the acceptance of a student into a school, but it is certainly a useful tool in setting a standard for one's school.

Many of the Allied Medical Professions have used selective tests for many years with evident success, why can not the Medical Technologists? Some of the pathologists during World War II used Aptitude Tests for eliminating applicants without the qualifications and found them a necessity. He asked me what areas I felt required a high degree of adaptability for a Medical Technologist. I explained that in my experience in organizing an approved school and dealing with students, that the important areas are, intelligence, scientific and mechanical aptitude, and last, but by no means the least, a good profile of personal and social adjustment.

He told me that a battery of tests would be necessary in order to measure these different specific abilities. He selected the ACE test, 1952 Edition, the Flanagan Aptitude Classification Battery, and the Guilford-Zimmerman Temperament Survey. Doctor supplied me with the personality tests but I purchased the ACE and the Flanagan Aptitude Classification Battery. The Flanagan Aptitude Classification Battery for a Biological Scientist consisted of four different type tests. These were Judgment-Comprehension, Scales, Components and Expression. At this time I just tried the one "Judgment and Comprehension," to see if the results paralled the ACE test. When purchasing Standardized

Tests there are many forms to be made out stating your qualifications, before the supplier will send them to you.

I administered these tests to my known class of students. I felt that this was necessary in order to set a "norm" by which to adhere when testing unknown students. This class of six had been in school for about six months. We were aware of their intellectual abilities and the good and bad points of their personalities. The results of these tests proved interesting and what is more important, they were valid. The results unfolded to me the student who was very intelligent but did not reach his capabilities, those with average intelligence but would complete the job in a longer period of time, the ones who needed extra help in mathematics, and those who just didn't possess the scholastic standing for the profession of Medical Technology.

There are two requirements of a good test, Validity and Reliability. Validity is defined as the degree to which a test measures what it is supposed to measure. Reliability occurs when the test yields consistent results if repeated, or if an alternate form of the same test is used.

The ACE test is an attempt to govern the scholastic aptitude of the student. The initials stand for the American Council of Education who are responsible for this cooperative test. It is composed of six type tests. They are Arithmetical Reasoning, Number Series, Figure Analogies, Same-opposite, Completion, and Verbal Analogy. The first three give the Q score, or measure quantitative abstract problem solving skills, while the last three give the L score and measure linguistic or verbal skill.

The Arithmetical Reasoning test has the obvious advantage of "Familiarity". It is particularly valuable in mathematics of the physical sciences where the stimulus appears in the form of a laboratory problem requiring computation. The completion test has a wide applicability as far as subject matter is concerned. The same opposite test also has the advantages of wide range of subject matter, and a wide sampling of knowledge tested per unit of working time. Figure and verbal analogies will bring out the student's Judgment, Comprehension and Spatial Relations. The ACE Battery is composed of practice problems preceding each test so that the student is familiar with the type. This is a time test and it should be adhered to accurately. The time for the completion of the test is one hour. There are more questions than one can answer in that length of time. The number of questions answered in a certain length of time in a specific type of test, determines the student's ability in a certain type of work. There are two-hundred possible points, eighty in the quantitative score, and one hundred and twenty in the linguistic score. I did not show the students the results of their in-

telligence test but I told them the results whether good, average, or poor.

The Guilford-Zimmerman Temperament Survey analyzed the personality and their opposites on the following points: General activity, Cooperativeness, Masculinity, Ascendance, Feelings of Inferiority, Talkitiveness, Depression, Emotional Stability and Subjectivity. Personality tests have no value if the student is not honest with himself or herself.

Their personality answer sheets were returned to the professor and he prepared the profiles and returned them to me. They resulted in real character sketches and so true. The profiles gave me the answers to many mysteries. I then had a conference with each student individually and permitted each one to view her own profile. I tried to point out their fine personality traits which determine good character and the ones which needed improvement by citing examples that occurred in their everyday life. The students were overwhelmed by the truthfulness which was exhibited in the results of these tests.

By knowing and studying these individual personality traits before admittance into the school, many opportunities will be afforded to guide and direct the students during their year of internship in proper attitude and behavior.

In our schools of Medical Technology we have a very serious obligation to teach the students not only theoretical and technical aspects of the profession but we must offer them an opportunity in the training of personality which will develop into good character. Some people possess these gifts naturally, others must acquire them, and still others possess them, but they are in a dormant state and must be brought to the surface by those who are responsible for their training.

There is a statement which I read a few years ago and I think it applies very well to our profession, "The Technical training will never compensate for an undesirable personality." I am sure that all of you have seen and encountered many skilled Medical Technologists, who, because of their disposition, were complete failures in their career. Their personalities were so disagreeable that no one could work with them.

I was also responsible at this time for an Approved School of X-ray Technology. The class consisted of four students. After they were examined, it was discovered that their scores paralleled those of the students in Medical Technology. The results were so accurate that the instructors wanted to take the test. All together now twenty students had been tested, including the new class just entering the school.

If a student applied from a distance, I sent the tests to one of their teachers for administration and then the tests were returned to me for scoring. There are several opinions on "Who

Can Administer Standardized Tests." Some authors say that only testing experts should administer these tests while others are of the opinion that anyone who can read well and follow directions can administer these tests in an accurate manner.

The four thousand Standardized Tests which are in use today are accompanied by manuals which give directions in an extremely explicit manner. Anyone of average intelligence, I feel, can administer group tests accurately, but only with experience in this type of work can interpretations of these tests be valuable.

After studying these results with the knowledge of the people tested I was able to set a standard score in which I could be guided in selecting students for our school. The required score on the ACE test was ninety points minimum. If the results fell below this, the applicant was rejected. The personality test was used to guide the individuals during their training period but not for elimination.

Now I felt that I was getting somewhere and, indeed, I needed this encouragement, because this testing program was new to me and only under the auspices of a testing specialist would I ever have dared to venture such an undertaking. Yet all the while I knew that for the sake of my profession and the individual human being interested in this work there had to be some way of knowing validly whether or not the applicant was suitable for the profession of Medical Technology.

At this time I was transferred to another hospital and was not able to see the results verified by the new class but from all reports these, too, proved valid.

In the hospital to which I am now assigned I have had the opportunity of conferring with the School of Medical Technology connected with the State University and Medical School. The director and instructors permitted me to administer the testing program to their known class of fifteen students. I used the ACE test and the California Personality Test on the entire group. The Flanagan Classification Battery was given to a few to see how the results correlated with the ACE test.

The California Personality Test measures several components other than those of the Guilford-Zimmerman Temperament Survey. The points which this test covers are Self-Reliance, Sense of Personal Worth, Sense of Personal Freedom, Feeling of Belonging, Freedom from Withdrawing Tendencies, Freedom from Nervous Symptoms, Social Standards, Social Skills, Freedom from Anti-Social Tendencies, Family Relations, School Relations, and Community Relations. This test is divided into two sections: the purpose of Section I is to indicate how the student feels and thinks about himself. Section II reveals how the student functions as a social being.

The Flanagan Classification Battery measures speed and ac-

curacy in reading scales, graphs and charts; it will show whether the student has the ability to read with understanding, to reason logically and to use good judgment in practical situations. It also measures the ability to identify important component parts and the feeling for and knowledge of correct English.

I would like to demonstrate the validity of these tests as shown by the comments of the teachers of the University School. May I read the following evaluations:

1. Total score was one hundred and nine of which twenty-six was the Q-score and the L-score being eighty-three. "Below average student. Has to work for what she gets. Not strong in mathematics as test shows."
2. The test showed that the total score was one hundred fifty-four. "We did not know she has the ability shown here, apparently does not study. We could have expected more all year had we known."
3. The results demonstrated a Q-score of forty-six points and a total of one hundred twenty-two points. "Average student. This agrees with performance she gives."
4. This student answered one hundred eighty-eight questions correctly out of possible two hundred which is very outstanding. "Every answer correct as far as she went. Did not finish, time ran out. Rather quiet, apparently was a little 'lost' with all the new work last summer, but did well. Now she is beginning to stand out above all the rest. Would make a good teacher."
5. The test resulted in one hundred thirty-seven questions answered correctly. "Thorough, good student. This grade agrees with our evaluation."
6. This student made one hundred twenty-one points total, with a Q-score of forty-nine. "Does better work than this shows; may work slower than average. Scoring guide showed that almost all answers were correct as far as the student went. Time did not allow better results."
7. Total score of one hundred twenty-nine points of which fifty-two were the Q-score. This shows greater ability. "Can do good work. Family problems have interferred with higher grades."
8. The test resulted in a total of ninety-six points. "This surprised us. The student does much better than this score would indicate, which must mean, (1) she is slow, (2) she works hard, or (3) she finds tests hard. Her personality, however, is very good, and she will do well when she gets out."
9. This student scored one hundred twenty points as a total.

"Average student. Could do better if she wanted to work. Social life probably interferes."

10. The total score made by this student was one hundred sixty-two points of which fifty-eight was the Q-score. "A bright 'A' student. Expected this grade. Does very good work."
11. The test resulted in one hundred six points as a total score. "Average student. Has tendency to be nervous, very cooperative."

The director of the University School is now in favor of giving these tests to the incoming class of students.

Summary

The purpose of this paper is to share my experience with those of you who wish to use Aptitude Tests in selecting your students of Medical Technology.

This is only a beginning of what might be accomplished in the future in testing potential students in our field.

I think Aptitude Testing is more necessary in "Hospital Schools" than in "University Schools." In the former the students do not always apply directly from college but their education is interrupted for a year or more, while in University Schools they are fairly weeded out before reaching their year of internship. These tests, however, would prove very beneficial to the instructors in counseling and guiding the students during training.

If the Scholastic score falls below ninety points I would be inclined to reject the student. The personality test is used primarily for guidance and counseling and has proven invaluable.

I would like to restate as I mentioned previously in this paper we are aware that every creature has a special work in life to do. If we can be of any assistance in helping an individual find his or her chosen profession, we have done a great charity. On this will rest the health and happiness of the individual and the superiority of our profession.

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TO DO OR NOT TO DO A COOMBS TEST*

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If one simple rapid and infallible procedure for cross-matching blood were devised, I'm sure most of us would breathe a sigh of relief. However, until some such test is available we must do the best we can with the tests now known to us. The Coombs test, in conjunction with the saline and albumin methods of crossmatching, seems to come nearest to giving us assurance that donor and recipient bloods are compatible.

In 1945, Coombs, Mourant and Race[1] showed by a new test—the anti-human globulin test—a method for demonstrating weak or incomplete blood group antibodies. Red cells sensitized by an incomplete (albumin active) anti-Rh serum, then washed free of the excess serum, were agglutinated after the addition of the serum from a rabbit previously immunized against human globulin. The rabbit anti-globulin serum causes the sensitized cells to be agglutinated. The sensitization depends upon the fixation of the antibody to the surface of the red cells which persist in spite of repeated washings of the red cells. It is interesting to note that soon after this test had been developed, Coombs discovered that Moreschi[2] in 1908 had used the same technique.

With the introduction of the anti-human globulin test into clinical work, its first application was for the detection of blood group antibodies, particularly Rh antibodies. Soon after, its use was extended to the diagnosis of acquired hemolytic anemia and identification of the rarer antibodies.

The Coombs antibody is not a single antibody but a multiplicity of antibodies. Dacie[3] was the first to consider that it consisted of at least two fractions. He showed that an anti-globulin serum neutralized with small amounts of gamma globulin failed to react with Rh-sensitized cells or with cells from patients with acquired hemolytic anemia of the "warm-antibody type." The serum would, however, react with cells sensitized by normal cold antibodies or with cells from patients with acquired hemolytic anemia of the "cold-antibody type." Previously Coombs and Mourant in 1947[4] demonstrated that the Rh incomplete antibody is mostly, if not entirely, in the gamma globulin fraction of serum.

Differences between the types of globulin on sensitized cells were demonstrated further in 1951 by Crawford and Mollison[5] who showed by cross-absorption experiments that an anti-globulin serum absorbed with cells sensitized with Rh antibody still

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will react with cells sensitized with normal incomplete cold-antibody. In 1952 Renton[6] separated the Coombs reagent into two fractions by absorbing it with suitably sensitized cells.

It is important to realize that the Coombs reagent does not react with a specific antibody, but rather with any human globulin which is attached to the cells. When washed red cells are agglutinated after the addition of Coombs serum, it is correct to assume there is an antibody coating the cells, the specificity of which can be determined only by testing the serum against normal bloods of known types.

Technically, the Coombs test appears to be a rather simple procedure—a matter of thoroughly washing the red cells under test to remove all circulating protein (that which is not attached to the red cells) and adding a drop of the Coombs reagent of adequate titer and specificity, following the particular directions prescribed by the manufacturer of the reagent used. Washing of the red cells before the addition of Coombs serum is of great importance because insufficient washing may result in either a false positive or a false negative result. The false negative result may occur if human globulin is present in excess of that which coats the cells. Adequate washing of the red cells removes this excess human globulin which, if present, neutralizes the anti-human globulin (Coombs reagent). The false positive result may occur on the basis of coagglutination.[7] The phenomenon of co-agglutination where red cells are clumped by mixtures of protein antigens and homologous anti-sera was originally described by Bordet and Gerzon. The system was demonstrated with unsensitized red cells and anti-globulin serum to their respective serum proteins, e.g., human red cells, human anti-globulin serum, horse-horse, rabbit-rabbit, etc. This pseudo-agglutination may complicate antiglobulin sensitization tests when cells are inadequately washed.

The Coombs test is used in two different ways. First, it is used to test the red cells of a patient to determine whether or not the cells have acquired a coating of antibodies. This is known as the direct Coombs test. Secondly, the Coombs test is used to test a serum, usually from a patient, for the presence of abnormal Blood Group Antibodies. In this instance, the serum under test is incubated with suspensions of normal red cells before the Coombs test is performed. This use of the Coombs test is therefore known as the indirect Coombs test.

The following are specific examples of passively acquired hemolytic anemia, the diagnosis of which will be confirmed by the performance of the Coombs test:

Erythroblastosis fetalis: A positive direct Coombs test on Cord Blood indicates that the red cells have been sensitized "in utero" by a maternally formed antibody. This is an important diag-

nostic test because it automatically establishes the diagnosis of erythroblastosis fetalis. However, a negative Coombs test does not exclude erythroblastosis, (especially in those cases caused by Anti-A or Anti-B).

Transfusion: A second example of passively acquired hemolytic anemia is that which may result when blood from a so-called "dangerous" Group O donor i.e. one whose titer of immune anti-A or anti-B agglutinins is given to a Group A or Group B recipient, as the case may be. About 30% of Group O individuals have immune anti-A and/or anti-B which may coat the red cells of a recipient, the same as anti-Rh. This may be avoided by using donor blood of the same type as the recipient whenever possible, or by selecting donors with low titers of iso-antibody.

In a similar fashion, a donor's serum may contain anti-Rh, anti-Kell, or other antibodies. This may occur more frequently than we realize because a large percentage of donors who were in the Military Service may have been transfused during that time. Such an incompatibility is not anticipated and would not be detected unless a minor crossmatch were done by the indirect Coombs technic. This is a strong argument against the use of Rh-negative blood indiscriminately, except in case of emergency.

Idiopathic Acquired Hemolytic Anemia: A positive direct Coombs test is obtained in most cases of idiopathic acquired hemolytic anemia. Not infrequently agglutinins are also present in the serum of a patient with this disease which result in an incompatible crossmatch with all available donors. Often this incompatibility is detectable only by the indirect Coombs crossmatch.

The use of the indirect Coombs test is illustrated by the recommended method for the selection of donors for patients idiopathic acquired hemolytic anemia. This method requires setting up serial dilutions of the patients serum against the cells of a number of prospective donors, one of whom should, if possible, be a known e-negative because of the frequency of anti-e in serums of patients with this disease.[8] In more and more cases, specific antibodies are being found in these patients which suggests the advisability of crossmatching them with a variety of donors known to be negative for the various blood group factors. If titer differences are noted with different donors it is presumptive evidence that there is at least one specific antibody in the patient's serum, and donors are selected accordingly. The donor who gives the lowest titer with the patient's serum should be used. The titrated crossmatches should be incubated for fifteen minutes at room temperature, centrifuged and examined for agglutination. Then the same tubes should be incubated for another fifteen minutes or more at 37 degrees C. and read again after centrifugation. They should then be converted to the albu-

min tube test by removal of saline, addition of albumin, and recentrifugation and examined after which they should be washed three times with saline and the Coombs reagent added.

The indirect Coombs test is a very useful and sensitive test for the detection of anti-Rh antibodies formed in the early stage of pregnancy. Certain other antibodies such as anti-K, anti-S, anti-Fy^a and anti-Jk^a are usually demonstrable only by the indirect Coombs test, and are known to have caused erythroblastosis fetalis and transfusion reactions. For this reason, the indirect Coombs test is becoming more widely accepted as an essential step in crossmatching bloods for transfusion. If done carefully and accurately, it should cut to a minimum the possibility of a hemolytic transfusion reaction.

Another important application of the indirect Coombs test is for the detection of the Du antigens. As you know, a cell is classified as a Du (which is a variant of D) when it is agglutinated by some anti-D reagents and not by others using the standard test methods. The calculated frequency of these antigens is approximately 1% in a random white population and up to 5% in negroes. Of what importance are the Du antigens? They can immunize a D negative recipient and anti-D, not anti-Du, may be produced; they can cause a transfusion reaction if the recipient is already immunized by D; and they can cause erythroblastosis fetalis if the mother is immunized by D.

The recommended procedure to insure, in so far as possible, the detection of Du's among donors is the indirect Coombs test (on all D negative bloods) using a potent incomplete anti-D serum. If the blood is a Du, a variety of results may be obtained —not always a strong reaction. The test for Du should under no circumstances be done on a patient. Where these D variants are concerned, we still have the double standard—a donor is called Rh-positive if he is a Du, or if there is any doubt about the result and a patient is called Rh-negative unless he is a definite strong Rh-positive.

The test for Du is much more likely to give a false positive result than the routine test for Rh, so one does not dare to type patients by this method. For example, the cells of a patient with acquired hemolytic anemia if coated with an antibody will give a positive direct Coombs test. If the cells of such a patient were to be typed or crossmatched using the indirect Coombs test technic, a false positive result would be obtained, because the cells, by virtue of the positive direct Coombs test, were shown to have been coated with an antibody. Therefore the importance of running a control (a direct Coombs test) is apparent.

Whether it is because so many more transfusions are being administered and more people are being immunized, or our

methods for detecting immunization are so much more sensitive it is difficult to say. However, the fact remains that the most comprehensive crossmatch possible should be done and even this combination of tests cannot be guaranteed to rule out serologic incompatibility.

In the opinion of our Laboratory we feel the minimum crossmatch should include either of the following procedures:

Method I

1. A major and minor crossmatch using saline suspensions of the cells, 15 minutes at room temperature, centrifuge and examine for agglutination. This step is most likely to pick up any ABO incompatibility.

2. Simultaneously, set up duplicate tubes placing two drops of the serum of patient and donor into appropriately marked tubes. By means of a wooden applicator stick remove a few cells from the patient's blood and suspend them in the donor's serum and a few from the donor's blood into the patient's serum, add one drop of 20% albumin to each tube, incubate at 37° C. for at least 30 minutes, longer is better, centrifuge and read. Convert these tubes to Coombs and examine microscopically.

Method II

Two major and one minor test tube crossmatches are done on every blood.

Tube #1: Patient's serum plus saline suspension of donor cells. Incubate 30 to 60 minutes at 37° C. Centrifuge, read and convert to Coombs.

Tube #2: Patient's serum plus serum suspension of donor cells. Incubate 30 to 60 minutes at 37° C. Centrifuge and read.

Tube #3: Donor serum plus patient's cells in own serum. Incubate 30 to 60 minutes at 37° C. Centrifuge, read and convert to Coombs.

A patient is always typed for A, B and D with every single crossmatch. It is essential to do an indirect Coombs crossmatch if the room temperature crossmatch is omitted.

We are frequently faced with the problem of what to do in the case of multiple transfusions to be given over a brief period of time. In addition to either Method I or II above, the serum from each donor should be further tested by the indirect Coombs test against a blood (or pooled bloods) containing all known antigens.

We have found that the rate at which tubes are centrifuged after the addition of Coombs serum is critical. If spun too hard, the manipulation required to resuspend the cell button may lead to a false negative reading unless examined microscopically. Another troublesome factor is using a too heavy cell suspension,

which may cause a false positive reading. Therefore, it cannot be emphasized too strongly the absolute need for following the printed instructions accompanying the reagent used.

It might be helpful to mention here one or two typing and crossmatching problems encountered occasionally.

1. An infant who has a positive direct Coombs is one of the commonest sources of confusion in the laboratory from this point of view. Such cells may be "blocked," i.e. so coated with maternal antibody that they do not react with and are not agglutinated by the anti-Rh typing reagent and therefore appear to be D negative. Should this occur it is reasonable to assume that if the mother's serum contains anti-Rh agglutinins then the baby with a positive direct Coombs must be Rh positive.

When selecting donor blood for an exchange transfusion the major crossmatch should be done using the mother's serum against the cells of the potential donor. Never use blood for a baby for an exchange transfusion that you would not give to the mother. If this rule is followed, no difficulty should be expected.

2. The cells of patients with cancer, myeloma, etc. whose serums contain the so-called straightforward auto-agglutinins usually give a negative direct Coombs test. These auto-agglutinins are strongest at 5° C and diminish at warmer temperatures. However, such a patient may have a positive direct Coombs if his blood is stored overnight at 5° C before being tested. Here a minor crossmatch by the indirect Coombs method serves as a control. Conversely, usually a patient who has a positive direct Coombs does not have an auto-agglutinin. Therefore, a major serum crossmatch can be performed. However, occasionally one does encounter a patient with both a positive direct Coombs and auto-agglutinins. In such a case, a fresh sample of blood should be drawn from the patient, using a warm syringe and then washing the cells in warm saline until no auto-agglutination is observed. These washed cells can then be used for any crossmatch and will serve as a control.

Summary

An attempt has been made to indicate how, in our opinion, the Coombs test, both direct and indirect, should be used in the Laboratory, some of the complexities of this test, and a brief discussion of crossmatching methods and problems.

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IN MEMORIAM

Dorothy Faber, Minnesota—August, 1956
Mary Goerl, Ohio—July, 1956
Betty Gow, Georgia—November, 1955
Ann Hellen, Maryland—June, 1956
Jurelle Hooper, Georgia—December, 1955
Florence Pettey, Texas—June, 1956
Lillian Scarberry, Kentucky—May, 1956
Lucille Wallace, Minnesota—October, 1956

All these—our friends—who gave so much of self, of talent, of loyalty and integrity—to strengthen our society and profession. We shall miss them. We must not grieve—for grief is selfish—but to keep the faith, we go forward to make our own contributions augment theirs. Let us be worthy of their example.

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